DEC 28 1961
BIOLOGY LIBRARY

VOLUME 2, NO. 3 NOVEMBER 1961

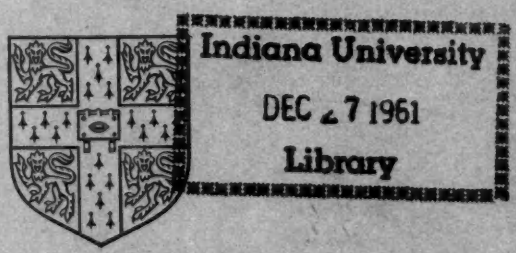
GENETICAL RESEARCH

EDITORIAL BOARD

C. H. WADDINGTON (*Chairman*)
CHARLOTTE AUERBACH D. LEWIS
H. GRÜNEBERG G. PONTECORVO
W. HAYES A. ROBERTSON

EXECUTIVE EDITOR

E. C. R. REEVE



PUBLISHED BY

CAMBRIDGE UNIVERSITY PRESS

Bentley House, 200 Euston Road, London, N.W. 1
American Branch: 32 East 57th Street, New York 22, N.Y.

PRICE 40*s*. net (U.S.A. $7.00)

GENETICAL RESEARCH

GENETICAL RESEARCH publishes original work on all aspects of genetics, or in any field of research which has an important bearing on genetics. Papers will be submitted to referees before acceptance. They will generally be printed in order of acceptance. In addition, short notes limited to 1500 words or the equivalent space including figures, etc., may be included if received 3 months before publication date.

EACH ANNUAL VOLUME will consist of three parts (to be published in February, July and November) plus an index.

SUBSCRIPTIONS may be sent to any bookseller or to the Cambridge University Press, 200 Euston Road, London, N.W. 1. Subscriptions in the U.S.A. should be sent to the American Branch of the Press, 32 East 57th Street, New York 22, N.Y. The subscription price is £5 net (including postage) for an annual volume of three parts ($17.50 in the U.S.A.), payable in advance; Separate parts 40*s*. or $7.00 each (plus postage).

NOTES FOR CONTRIBUTORS

CONTRIBUTIONS will be welcomed from scientists of all nationalities, but must be written in English, with an English summary. An additional summary in French, German, Russian or Italian will be accepted. Papers should be sent to the Executive Editor, Dr. E. C. R. Reeve, Institute of Animal Genetics, West Mains Road, Edinburgh 9.

GENERAL. Submission of a paper or note will be taken to imply that it is unpublished and is not being considered for publication elsewhere. Papers published in *Genetical Research* may not be reprinted or published in translation without permission from the Editorial Board. Papers should be as concise as clarity permits, and illustrations kept as few as possible. Attention to the following instructions will assist rapid publication.

TYPESCRIPTS. A top copy and one carbon copy should be submitted. They should be typed with double-spacing, on one side of the paper, leaving margins of about 1½ inches at the left-hand side and head of each sheet. Quarto is preferred to foolscap paper. Heavily corrected typescripts may have to be returned to the author for retyping. Papers should state the name and address of the author (or his proxy) to whom proofs are to be sent. The address of the laboratory at which the work was carried out will be printed with the authors' names at the head of the paper, and changes of address and sources of financial support may be added in footnotes.

A short title should be supplied for running headlines; this must not exceed 50 characters, counting each letter and space as one character.

(*continued inside back of cover*)

Genet.
With
Print

Stu

The
reas
testi
of m
of w
effec
com
gene
use
T
man
Fish
whil
mut
com
of n
com
yet
bilit
mat
T
195
cell
deri
a F
The
sing
of t
par
occ
pro

Genet. Res., Camb. (1961), **2**, *pp.* 309-332
With 7 text-figures and 2 plates
Printed in Great Britain

Studies on histocompatibility mutations in mouse tumour cells using isogenic strains of mice

By S. S. DHALIWAL*

Institute of Animal Genetics, West Mains Road, Edinburgh 9

(*Received* 24 *June* 1960)

INTRODUCTION

The study of chemical mutagenesis in mammals has been limited for a number of reasons. The long interval between generations and the technical difficulties of testing large numbers of animals for quantitative mutation work make the study of mutations in higher organisms almost impossible. The treatment with mutagens of warm-blooded animals, e.g. mice, at doses high enough to produce genetical effects usually also produces severe systemic effects which make the tests of the compounds difficult. Most of these difficulties are overcome by testing for mutagenesis at the cellular level, either *in vitro* or *in vivo*. This is made possible by the use of tissue culture and tissue transplantation.

Tissue culture has been used fairly extensively to study the genetics of somatic mammalian cells *in vitro* (Puck, 1957, 1958*a*, *b*; Puck & Marcus, 1955; Puck & Fisher, 1956; Puck & Cieciura, 1958; Puck *et al.*, 1956, 1957). Tissue culture, while having certain advantages for quantitative work, is not ideal for chemical mutagenesis as the physiological environment in which the cells are growing is completely different from that found *in vivo*. Tissue transplantation, especially of neoplastic cells, is extremely useful. The transplantation of neoplastic cells in compatible hosts provides a condition in which the cells can divide rapidly and yet maintain their genetical stability over a number of generations. The availability of histocompatibility markers in mouse neoplastic cells provides a useful material for studying mutagenesis in these cells.

The development of isogenic resistant (IR) strains of mice by Snell (1948, 1953, 1955) provides a useful marker in studying cellular variation in mouse tumour cells. An IR strain of mice is genetically similar to the strain from which it is derived except for the histocompatibility-2 (H-2) locus. A tumour originating in a F_1 hybrid between two IR strains is homozygous at all loci, except the H-2 locus. The F_1 tumour is not transplantable to either of the parental strains. However, a single mutation at the H-2 locus towards one of the parental strains or a loss of one of the H-2 alleles in a hybrid tumour cell would make it compatible with that parental strain. It is, thus, theoretically possible to detect a single mutation occurring in a single cell of a large neoplastic population. Such a system was proposed by Lederberg (1956) and used by Mitchison (1956), Klein, Klein &

* Present address: Department of Zoology, University of Malaya, Kuala Lumpur.

Révész (1957) and Klein & Klein (1958, 1959). The aim of the present study is to see the feasibility of using such a system to test various mutagens and carcinogens for their mutagenic activity in mouse tumour cells.

Tumours induced in F_1 hybrids were tested in the parental strains to see if they would produce variants compatible with the parental strains. Hybrid tumours which produced variants regularly in the parental strains were tested after treatment with X-ray or triethylenemelamine to see if the production of variants could be increased by using these mutagenic agents. An increase in variant production by known mutagens would support the hypothesis that these variants are due to mutations at the H-2 locus.

MATERIALS AND METHODS

Mice

The strains of mice used were Snell's A strain and its two isogenic resistant sublines A.SW and A.CA, obtained from Prof. G. Klein, Karolinska Institutet, Stockholm. All three strains have a strain A background and were developed by Snell (1948, 1953, 1955). Snell's original A line is genotypically H-2a/H-2a, while its isogenic resistant sublines, A.SW and A.CA, are H-2s/H-2s and H-2f/H-2f, respectively (Snell, 1955; Allen, 1955). For convenience the H-2 genotype of the three strains will be referred to as a/a, s/s and f/f.

Tumours

Eighteen sarcomas were induced in hybrid mice (A × A.SW, H-2 genotype a/s) with 20-methylcholanthrene. The tumours were maintained by serial passage in hybrid mice. Most of the tumours were frozen in four parallel tubes, either immediately after the origin of the tumour or after a few passages in hybrid mice, and stored at −79°C. The tumours were numbered as.1, as.2, etc., indicating the H-2 genotype of the hybrid tumour. The tumours were transplanted subcutaneously by trocar and canula into the right flank or bilaterally.

To inject a known cell dosage, a suspension of the tumours in Ringer's solution was prepared by pressing them through a 60-mesh stainless steel sieve.

The cells were counted in a Buerker haemocytometer. The live cells were differentiated from dead cells by staining with 1% Nigrosin in Tyrode solution (Kaltenbach, Kaltenbach & Lyons, 1958). Cell counts were based only on unstained cells.

Treatment of cell suspensions with triethylenemelamine (TEM) and X-rays

Cell suspensions prepared as above were divided into two equal parts. One part was left standing at 3°C. while the other was subjected to the treatments.

The tumour cell suspensions were treated with TEM dissolved in sterile water at concentrations of 10 γ/c.c. (1:100,000) or 20 γ/c.c. (1:50,000). The treatments were done for 2 hours at 3°C. After treatment the cells were centrifuged and washed twice with Ringer's solution.

Cell suspensions were X-irradiated in pyrex centrifuge tubes at a distance of 250 mm. from the X-ray tube. The X-rays were generated at 140kV and filtered through 1 mm.Al. Doses of 150 r to 500 r were administered at a rate of 56·92 r per minute.

After treatment the cells were recounted in a Buerker haemocytometer after staining with 1% Nigrosin, and were brought to the required concentration. The percentage of unstained to stained cells varied between 15 and 25. It was found that the percentage of unstained cells to stained cells did not change after treatment with TEM or X-rays.

Mice inoculated with tumours were palpated regularly every third day after inoculation. Records of regression of the tumours were kept. Mice which failed to produce tumours were left for 3 months before they were killed and scored as negatives.

Cytological preparation of tumours

The tumours were prepared for cytological examination according to the method of Bayreuther & Klein (1958). The prepared pieces of tumour were stored in 45% acetic acid at −15°C. Permanent preparations were made by squashing small pieces of tumour on an albuminized slide and passing them through the alcohol series and mounting in Xam.

RESULTS

Transplantation tests with hybrid tumours ($A \times A.SW$ F_1, genotype a/s) induced with 20-methylcholanthrene

Fourteen hybrid tumours (H-2 genotype a/s) were tested in various numbers of parental mice, usually after the first transfer generation (t.g.). Some tests had to be delayed till after a few passages in hybrid mice. The results of the pooled transplantation tests are summarized in Table 1. These agree with those of Klein & Klein (1958, 1959). There is a large amount of variation in transplantation behaviour between individual tumours. It is, therefore, necessary to select carefully the tumours which are to be used in mutagenesis experiments.

Seven tumours behaved according to expectation: they grew only in the hybrid mice in which the tumour originated and gave no variants towards either of the parental strain.

Five tumours (as.1, 6, 7, 14 and 16) gave variants, occasionally or regularly, towards one or both of the parental strains. Table 2 gives the results of the transplantation tests with various variants that were isolated from hybrid tumours transplanted into parental mice. The variants bear the number of the original tumour followed by the number of the variant and the strain in which it originated. A.SW strain is designated as SW and strain A.CA as CA. In the production of the variants, the tumours always regressed completely at about 10–14 days after inoculation, and the variants then began to appear between one and two months after inoculation. Some of the tumours gave 'true variants' while others

gave 'false variants' which grew in mice of foreign genotypes and failed to grow in pre-immunized mice (see Klein & Klein, 1959).

Tumour as.7 was found to be the most interesting one and was extensively studied. It grew in 35·3% of A.SW mice and failed to grow in mice of the other parental strain or mice of foreign genotypes (Table 1). Six independent A.SW

Table 1. *Transplantation tests with various sarcomas induced by 20-methylcholanthrene in A × A.SW F_1 hybrids (H-2 genotype a/s)*

Host strain (and H-2 genotype)

Number of mice killed by tumour/Total number inoculated

Tumour no. and genotype	A × ASW F_1 (a/s)	A(a/a)		A.SW(s/s)		A.CA (f/f)	Other unrelated strains
		Untreated	Immunized	Untreated	Immunized		
as.1	22/22	1/22	0/16	2/22	0/24	0/6	0/12
2	0/6	0/10	—	0/12	—	—	—
3	12/12	0/10	0/10	0/13	0/26	—	—
4	12/12	0/12	0/12	0/10	0/10	—	—
5	12/12	0/24	—	0/24	—	—	—
6	10/10	0/10	—	1/12	—	—	—
7	96/96	0/81	0/24	36/102	2/26	0/20	0/10
8	11/11	0/12	—	0/12	—	—	—
9	11/11	0/13	0/12	0/12	0/12	—	—
10	12/12	—	—	0/12	—	—	—
14	14/14	2/12	—	5/12	—	0/12	—
15	6/6	0/12	—	0/12	—	—	—
16	13/13	1/6	—	0/10	—	—	—
17	14/14	0/18	0/6	0/14	0/6	—	—

variants of this tumour originating at different times were tested. They grew in 100% of the untreated and pre-immunized A.SW mice while failing to grow in A or A.CA mice (Table 2). One of the variants grew in 10/13 A × A.SW F_2 mice while another variant grew in 23/28 A × A.SW F_2 mice. Only one variant (as.7/2SW) produced a single growth in A.CA mice. This was found to be completely non-specific on further testing (Table 2). Tumour as.7 was also tested in 26 A.SW mice pre-immunized against this tumour. It grew only in 2 out of 26 (7·7%) of the pre-immunized mice.

Tumour as.14 was the only tumour that gave true variants towards both the parental strains, A and A.SW. The A variants grew in 100% of pre-immunized A strain mice and failed to grow in A.SW mice or mice of foreign genotypes. The A strain variants have remained specific over three transfer generations, thus showing them to be true variants for the A strain. The A.SW variant also failed to grow in mice of the other parental strain or of foreign genotypes and grew in a high proportion (13/18) of pre-immunized A.SW mice (Table 2).

Tumour as.2 failed to grow in mice of the parental strains as well as the hybrid mice in which the tumour originated. In all the mice inoculated, this tumour regressed after about 10 days, suggesting that the regression was due to the homograft reaction of the host. It is, therefore, possible that a mutation towards a

Table 2. *Transplantation tests with various variants originating from hybrid sarcomas (H-2 genotype a/s)*

Host strain (and H-2 genotype)
Number of mice killed by tumour/Total number inoculated

	A(a/a)		A.SW(s/s)					
Variants	Untreated	Immunized	Untreated	Immunized	A.CA (f/f)	Other unrelated strains	A × A.SW F_2	A.SW × A.CA (s/f)
as.1/1A	18/8	0/16	6/6	—	10/12	—	—	—
as.1/1SW	0/10	—	14/14	12/13	0/11	—	11/16	—
as.1/CA*	—	—	4/4	—	8/9	4/8	—	—
as.6/1SW	1/12	—	8/8	5/7	0/14	—	—	—
as.7/1SW	0/18	—	15/15	6/6	0/13	0/6	10/13	5/5
as.7/2SW	0/12	—	9/9	7/7	1/6	—	—	—
as.7/3SW	0/12	—	4/4	6/6	0/12	—	—	—
as.7/6SW	0/10	—	2/2	6/6	0/6	—	—	—
as.7/8SW	0/10	—	4/4	6/6	0/12	—	23/28	—
as.7/10SW	0/12	—	2/2	6/6	0/6	—	—	—
as.7/CA†	4/6	—	—	—	6/6	6/8	—	—
as.14/1A	2/2	12/12	0/6	—	0/6	0/5	—	—
as.14/1SW	0/8	—	2/2	13/8	0/7	0/5	—	—
as.16/1A	2/2	3/6	6/6	—	5/6	—	—	—

* Variant from A.CA host inoculated with variant as.1/1A.
† Variant from A.CA host inoculated with variant as.7/2SW.

different H-2 allele had taken place, most likely in the mouse in which the tumour originated.

Three of the original tumours, as.1, as.4 and as.7, became completely non-specific in the course of their passage in hybrid mice. The behaviour of these tumours after losing transplantation specificity is summarized in Table 3. In contrast to true variants originating in test mice, non-specific tumours failed to regress completely, although occasionally their growth remained retarded between 10 and 20 days after inoculation. Except for tumour as.4, the non-specific tumours failed to grow in pre-immunized mice. All these non-specific tumours were discarded in the study of the production of true variants towards the parental strains. In the case of tumour as.7, a subline derived from 10^2 cells at its fifth transfer generation in A × A.SW F_1 mice was used instead. This subline has been carried

Table 3. *Transplantation tests with three hybrid (A × A.SW F_1) sarcomas that became non-specific after a number of transfer generations (t.gs.) in A × A.SW F_1 mice*

Host strain (and H-2 genotype)
Number of mice killed by tumour/Total number inoculated

Tumour no. and genotype	No. of t. gs. in A × ASW F_1	A(a/a)		A.SW(s/s)		A.CA (f/f)	Other unrelated strains
		Untreated	Immunized	Untreated	Immunized		
as.1	5	9/12	0/16	0/16	0/18	5/6	0/5
4	4	22/22	6/6	20/20	4/4	12/12	—
7	7	15/31	0/6	16/26	0/6	11/13	4/5

to its tenth transfer generation in hybrid mice and has remained very specific, growing only in hybrid A × A.SW F_1 mice, giving approximately 30% variants towards the A.SW strain and failing to grow in A and A.CA strains or strains of foreign unrelated genotypes.

Titration of mouse sarcoma cells for viability after treatment with triethylenemelamine (TEM) or X-rays

As a preliminary to studying the mutagenic effects of various physical and chemical mutagens on tumour cells, it is necessary to work out a proper dosage for treating the cells. This could be done by working out the survival of the tumour cells at various doses of treatment. Survival or viability tests are also essential for calculating mutation rates per surviving cells rather than per treated cells.

Survival of treated tumour cells was calculated by titrating serial tenfold dilutions of the untreated and treated cells in groups of compatible hybrid mice. Four to eight mice were used per dilution. This meant the use of large numbers of mice. As an alternative the chorioallantoic membrane (CAM) of a developing chick embryo was used. Grafts of mouse tumours are known to grow on the CAM until the eighteenth day of incubation (Murphy, 1912).

For CAM titrations, embryos at the tenth day of incubation were used, and 0·1 c.c. of the cell suspension was inoculated on the CAM close to a large blood-vessel. The CAMs were cut open and spread out on a Petri dish. They were examined under a binocular microscope against a dark background. Membranes with distinct tumours were counted as positive while membranes in which no tumours could be seen under the binocular microscope were considered as negatives.

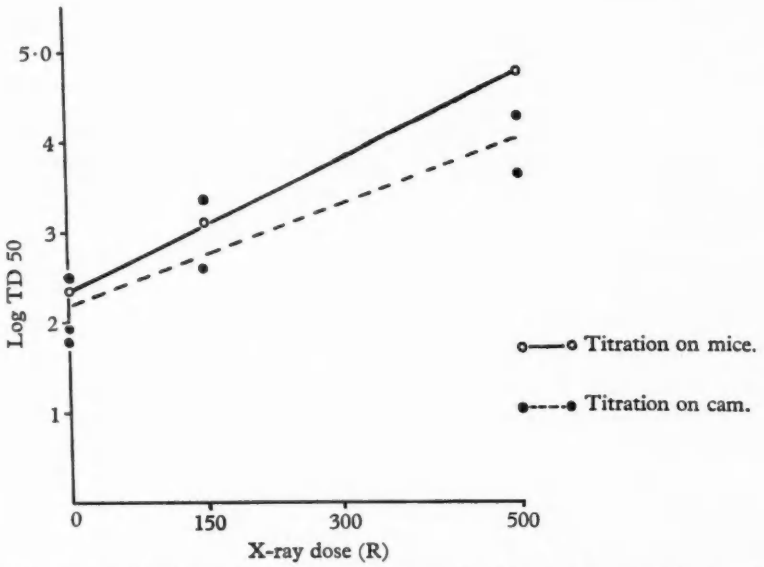
The TD_{50} (50% end-point) for the untreated and treated series was calculated according to the method of Reed & Muench (1938). Table 4 gives the TD_{50} for the untreated, X-irradiated and TEM-treated cell suspensions obtained by titrating in mice and on the CAM of chick embryos. In Text-fig. 1 the log TD_{50} is plotted against the X-ray dose. The values obtained from titration on mice fall on a straight line. A lower TD_{50} value has been obtained in most experiments from CAM titrations than from mouse titrations. This could be due to the failure to distinguish true tumours from non-specific lesions (which also appeared on control membranes without any inoculation). Non-specific lesions are due to the damage produced

Table 4. *TD_{50} for the untreated, X-irradiated and TEM-treated cell suspensions of tumours as.7 and as.1 titrated in mice and on the CAM of chick embryos*

Tumour no. and genotype	Titration on	Untreated	X-irradiated		TEM-treated	
			150 r	500 r	10γ	20γ
as.7	Mice	$10^{2\cdot35}$	$10^{3\cdot07}$	$10^{4\cdot75}$	—	—
as.7	Mice	$10^{2\cdot31}$	—	—	$10^{3\cdot25}$	$10^{3\cdot71}$
as.7	CAM	$10^{1\cdot83}$	—	$10^{3\cdot64}$	—	—
as.7	CAM	$10^{2\cdot46}$	$10^{3\cdot33}$	—	—	—
as.7	CAM	$10^{1\cdot92}$	$10^{2\cdot56}$	$10^{4\cdot25}$	$10^{2\cdot84}$	—
as.1	CAM	$10^{2\cdot17}$	$10^{3\cdot22}$	—	—	—

on the CAM or are a reaction on the part of the membrane to bits of shell dropping on it during inoculation. Titrations in mice appear to give better and more consistent results than titrations on CAM.

It must be noted that in all the experiments the TD_{50} for untreated tumour cell suspension lies in the region of 10^2 cells. In the untreated titration on mice no tumours were produced in twenty-four mice inoculated at a dilution of a single cell. One tumour was produced out of fourteen mice inoculated at a dilution of 10 cells. This suggests that a single viable cell is unlikely to give rise to a solid tumour when inoculated subcutaneously.



Text-fig. 1. Relationship between X-ray dose and log TD_{50} obtained by titrating tumour cell suspensions on mice and CAM.

The number of cells inoculated in the present case is based on the number of unstained cells. Recently, there have been a number of reports suggesting that not all stained cells are metabolically dead or inviable (B.E.C.C. Annual Report, 1951; Kaltenbach, Kaltenbach & Lyons, 1958). While the proportion of unstained cells could be used as an indication of the dilution range that should be titrated, the final results are best based on the total cell count.

Transplantation tests with 20-methylcholanthrene-induced hybrid sarcomas after treatment with triethylenemelamine and X-rays

Some of the hybrid tumours were selected in order to study the effects of triethylenemelamine (TEM) and X-rays on the transplantation behaviour of these tumours. These included tumours which were specific for the hybrid mice and

which gave variants regularly towards one of the parental strains. TEM and X-rays are the most powerful mutagens known and, as a preliminary, the investigation was limited to these two mutagens.

Cell suspensions of the tumours were prepared and treated with TEM and X-rays as described earlier. X-ray doses of 150 r, 250 r and 500 r were administered. TEM treatment was done at a concentration of 1 in 100,000 or 10 γ of TEM/c.c. of cell suspension for 2 hours at 3°C. The untreated controls and treated cell suspensions were inoculated into mice at the same time.

Table 5 summarizes the results of transplantation tests with tumour as.7 after treatment with TEM or X-rays in five different experiments. Tumour as.7, as already described, gave variants which were compatible with the A.SW parental strain but failed to give any variants towards the other parental strain (A). In experiments I to IV the cell suspensions were treated with TEM or X-rays *in vitro*, while in experiment V the tumour was treated with TEM *in vivo*. In experiments I to IV the untreated cell suspension of the tumour gave variants towards the A.SW strain in a percentage of mice ranging from 34·6 to 41·7. The average for the four experiments is 36·8%. The untreated cell suspensions of tumour as.7 failed to give any variants towards the A strain in all the experiments.

In experiments I to IV aliquots of the same cell suspension of tumour as.7 kept as untreated controls were treated with TEM at a concentration of 1 in 100,000 or 10 γ of TEM/c.c. of cell suspension. With the exception of experiment I, all experiments gave comparable results (Table 5). The percentage of variants isolated in the parental strain A.SW was significantly increased in the TEM treated series over the parallel untreated controls. The percentage of variants produced after treatment with TEM ranged from 57·1 to 61·5 for the three experiments, while the range for the untreated controls in the same three experiments was 34·6% to 37·5%. In experiment I, although there was no increase in the percentage of variants produced in A.SW mice per treated cells, there was obviously an increase per surviving cells after treatment. This experiment was also unique in that variants were produced after treatment with TEM towards the other parental strain in 4 out of 13 mice in which the tumour was tested (Table 5).

In experiments III and IV (Table 5) cell suspensions of tumour as.7 were treated with X-rays at a dose of 150 r for experiment III and 250 r for experiment IV. Again, the percentage of variants produced towards the A.SW strain was significantly increased after X-irradiation above those produced by untreated cell suspensions. In experiment III the percentage of takes in A.SW mice was increased from 35·7 with untreated cell suspension to 50·0 after treatment with 150 r X-rays, while in experiment IV the percentage of takes in A.SW mice was increased from 34·6 in the untreated series to 61·1 after treatment with 250 r X-rays. It thus appears that treatment with X-rays *in vitro* increases the number of variants produced and that 250 r is more effective than 150 r. No variants were produced in the A strain after treatment with 150 r or 250 r X-rays.

The above results with tumour as.7 show that TEM treatment and X-irradiation are effective agents in increasing the percentage of variants. Comparable cell

Table 5. *Transplantation tests with tumour as.7 without treatment and after* in vitro *treatment with TEM and X-rays in experiments I–IV. In experiment V the treatment with TEM was done* in vivo. *In parentheses are given the percentage of mice killed by progressively growing tumours*

Host strain (and H-2 genotype)

Expt. no.	Untreated			X-ray-treated			TEM-treated		
	Number of mice killed by tumour/Total number inoculated								
	Cell dosage	A(a/a)	A.SW(s/s)	Cell dosage	A(a/a)	A.SW(s/s)	Cell dosage	A(a/a)	A.SW(s/s)
I	$3{\cdot}5 \times 10^6$	0/12	5/12 (41·7)	—	—	—	$2{\cdot}5 \times 10^6$	4/13 (30·8)	4/11 (36·4)
II	$3{\cdot}8 \times 10^5$	0/28	9/24 (37·5)	—	—	—	$3{\cdot}0 \times 10^5$	0/24	6/10 (60·0)
III	$9{\cdot}6 \times 10^5$	0/13	5/14 (35·7)	$9{\cdot}6 \times 10^5$*	0/14	7/14 (50·0)	$7{\cdot}6 \times 10^5$	0/11	8/13 (61·5)
IV	$6{\cdot}3 \times 10^5$	0/28	9/26 (34·6)	$6{\cdot}3 \times 10^5$†	0/28	11/18 (61·1)	$6{\cdot}1 \times 10^5$	0/14	8/14 (57·1)
Total for expts. I–IV		0/81	28/76 (36·8)		0/42	18/32 (56·3)		4/62 (6·45)	26/48 (54·2)
V	$2{\cdot}9 \times 10^5$	0/12	4/12 (33·3)	—	—	—	$2{\cdot}9 \times 10^5$	0/12	0/12

* X-ray dose = 150 r.
† X-ray dose = 250 r.

doses were inoculated in the untreated controls and the treated series. Hence, both TEM and X-rays are effective in increasing the percentage of variants per treated cells. A proportion of the cells are killed as a result of the treatment. To estimate the actual effectivity of the agents used a correction has to be made for this. However, this correction is not a straightforward one in the above case as both the compatible and incompatible cells among the survivors and the untreated controls divide an unknown number of times before the homograft reaction sets in and selectively destroys the incompatible cells.

As the main interest in the present study was in chemical mutagens, only variants isolated in strains A and A.SW after treatment with TEM were tested further. The behaviour of these variants is summarized in Table 6. Three variants from each strain were tested. Two variants isolated in A.SW mice were specific for strain A.SW, growing in untreated A.SW as well as a high percentage of pre-immunized A.SW mice, and failing to grow in the other parental strain or mice of

Table 6. *Transplantation tests with variants of tumour as.7 isolated in strains A and A.SW mice after treatment* in vitro *with TEM*

Host strain (and H-2 genotype)

Number of mice killed by tumour/Total number inoculated

	A(a/a)		A.SW(s/s)			
Variant	Untreated	Immunized	Untreated	Immunized	A.CA (f/f)	Other unrelated strains
as.7/4SW	0/12	—	6/6	5/9	0/6	—
as.7/5SW	0/12	—	4/4	5/6	0/12	0/6
as.7/7SW	—	—	4/4	6/6	2/12	1/6
as.7/1A	9/9	6/6	0/6	—	0/12	—
as.7/2A	10/10	5/6	3/6	—	0/12	—
as.7/3A	4/4	6/6	4*/12	—	—	0/6

* One of the four tumours was tested further in seven A.CA mice and grew in five of them.

foreign genotypes (A.CA and unrelated strains). The other variant gave a proportion of takes (2/12) in A.CA mice and in mice of unrelated strains (1/6). Three of the four variants produced in strain A after treatment with TEM were tested. Only one of these three variants was found to be specific for the A strain (Table 6). It failed to grow in the other parental strain A.SW and grew in pre-immunized mice of the A strain. The second variant grew in a proportion (3/6) of the A.SW mice but in none of the A.CA mice (0/12). This variant was, therefore, not completely specific for A strain. The third variant gave 4 tumours out of 12 mice of strain A.SW in which the variant was tested. One of the four tumours was further tested in A.CA mice and was found to grow in 5 out of 7 mice. This variant appears to be non-specific to a large extent.

In experiment V (Table 5) the tumour cells were treated *in vivo* with TEM as follows. Four hybrid mice ($A \times A.SW\ F_1$) were inoculated with tumour as.7. After 10 days when the tumours were of reasonable size, two mice were injected intraperitoneally with a solution of TEM at a dose of 2 mg./kg. body weight. Two inoculations of TEM were done on two consecutive days. Two mice with

tumours were left untreated. Twenty-four hours after treatment, the tumours from the treated and untreated hybrid mice were removed and tested in the parental strains as shown in Table 5. The percentage of unstained cells in tumours from the untreated mice was 28·5, while in tumours from the treated mice it was 15·6. The untreated tumours gave variants in 33·3% of A.SW mice and none in the A strain, while in contrast to treatment *in vitro* the treated tumours gave no variants in either of the parental strains. This could be due to the fact that very few viable cells were present in the treated tumours. Moreover, the tumours would receive an uneven treatment and parts of the tumour receiving an effective dose of TEM would also be the parts that would be killed by the chemical.

In order to detect if a hybrid tumour could be changed towards a new H-2 allele, tumour as.7 was tested after treatment with TEM or X-rays in hybrid mice between A and A.CA and A.SW and A.CA (Table 7). As was found by Klein & Klein (1958), no true variants, specific only to these hybrids, could be isolated.

Table 7. *The top part of the table gives results of transplantation tests with tumour as.7 in A × A.CA F_1 and A.SW × A.CA F_1 mice after treatment with TEM and X-rays. The bottom part gives the results of transplantation tests with the variants which were tested without any treatment*

Host strain (and H-2 genotype)

Number of mice killed by tumour/Total number inoculated

	A × A.CA F_1 (a/f)		A.SW × A.CA F_1 (s/f)	
Tumour	TEM-treated	X-irradiated	TEM-treated	X-irradiated
as.7	1/7	0/6	1/6	7/14
as.7/A.CA (variant from A × A.CA)	A(a/a) 0/10	A × A.CA (a/f) 0/6	A.SW(s/s) —	A.SW × A.CA (s/f) —
as.7/S.CA (variant from A.SW × A.CA)	—	—	6/6	7/7

In addition to tumour as.7, three other tumours were tested in the parental strains after treatment with TEM or X-rays. Two of these tumours (as.3 and as.17) were very specific and had given no variants towards either of the parental strains. They failed to give variants after treatment with TEM or X-rays (Table 8). Tumour as.1 had given variants earlier spontaneously towards the A.SW strain. This tumour failed to give any variants when irradiated with 500 r. This may be due to the high X-ray dose used which would kill a large proportion of the tumour cells.

Cytological analysis of hybrid (A × A.SW F_1) sarcomas and their derived variants

Cytological analysis was carried out on two of the original hybrid tumours, as.7, and their variants isolated in mice of the parental strains. Chromosome counts were made by drawing roughly each metaphase on a piece of paper and counting the number of chromosomes from the paper. Checks were made on each metaphase

Table 8. *Transplantation tests with three hybrid tumours without treatment and after treatment* in vitro *with X-rays and TEM*

Host strain (and H-2 genotype)

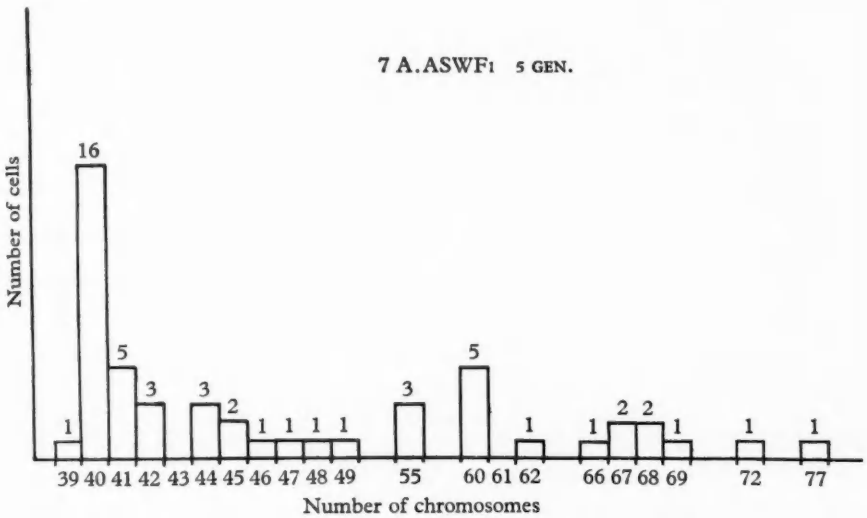
Number of mice killed by tumour/Total number inoculated

Tumour no. and genotype	Untreated			X-irradiated, 500 r			TEM-treated, 10 γ		
	Cell dosage	A(a/a)	A.SW(s/s)	Cell dosage	A(a/a)	A.SW(s/s)	Cell dosage	A(a/a)	A.SW(s/s)
as.1	$1{\cdot}8 \times 10^6$	1/22	2/22	$1{\cdot}8 \times 10^6$	0/24	0/24	—	—	—
as.3	$1{\cdot}7 \times 10^6$	0/10	0/13	$1{\cdot}5 \times 10^6$	—	0/11	—	—	—
as.17	$1{\cdot}1 \times 10^6$	0/8	0/14	—	—	—	$9{\cdot}0 \times 10^5$	0/13	0/12

by counting and recounting the chromosomes directly under the microscope with a tally counter. While the majority of the counts were exact, metaphase plates showing an error of more than ± 3 chromosomes were not included.

Tumour as.7

The cytology of tumour as.7 at its fifth transfer generation in hybrid (A × A.SW F_1) mice was examined. Fifty metaphases were examined and the chromosomes counted. As can be seen from Text-fig. 2, this tumour had a diploid modal number (40 chromosomes) as well as a few cells around the triploid number (60). Most of



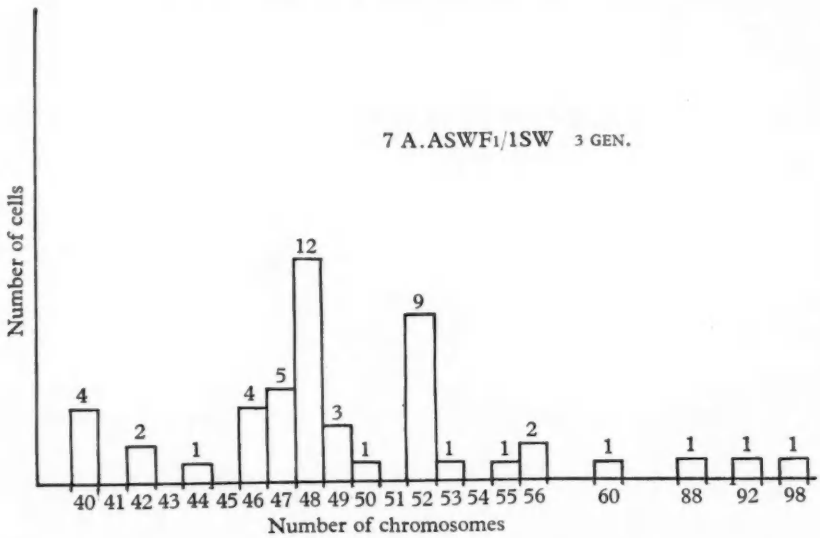
Text-fig. 2. Chromosome number distribution of tumour as.7 at its sixth generation, based on fifty metaphases.

the cells counted were exactly diploid (Plate I, Fig. 1), while a few cells were also found to be hyperdiploid. A small number of cells were triploid or hypertriploid and only 2 cells out of 50 counted had a hypotetraploid number of chromosomes. It may be of significance to point out here that this subline of tumour as.7 became non-specific at its seventh transfer generation in hybrid mice. It is possible that this could be due to the triploid cells, which were already present as a minority at the fifth transfer generation of the tumour, replacing the modal diploid cells. No marker chromosomes were regularly encountered and hence no detailed idiograms were drawn.

Variant as.7/1SW

This variant was isolated from an A.SW mouse inoculated with tumour as.7 at its first transfer generation in hybrid mice. The variant was maintained by regular transplantation in A.SW mice. The cytology of this variant at its third

transfer generation was examined. The distribution of chromosome numbers based on fifty metaphases is given in Text-fig. 3. Compared with the original tumour there appears to be a shift in the modal number from exactly diploid in the original tumour to hyperdiploidy. There appear to be two modes—one at 48 chromosomes (Plate I, Fig. 2) and another one at 52 chromosomes. There are fewer cells at the triploid number of chromosomes compared with the original tumour. This could be due to the early isolation of the variant from the original tumour compared with when it was used for cytological examination.



Text-fig. 3. Distribution of chromosome number of the variant 1SW, third transfer generation, of tumour as.7 which originated spontaneously. Examination of fifty metaphases.

Variant as.7/4SW

This variant was derived from an A.SW strain mouse inoculated with a cell suspension of as.7 at its third transfer generation after treatment with TEM. Cytological examination of this variant was carried out after its second passage in A.SW mice. This variant also has two modal numbers of chromosomes—one at 42 and another at 60 (Text-fig. 4, Plate II, Fig. 3). Compared with variant as.7/1SW which originated spontaneously, this variant has a larger proportion of cells with chromosome counts approximating to the triploid number.

Variant as.7/2A

This variant was also isolated from tumour as.7 which was treated with TEM at its third transfer generation. The variant was not completely specific for the A strain (Table 6). Cytological analysis of the variant at its second transfer

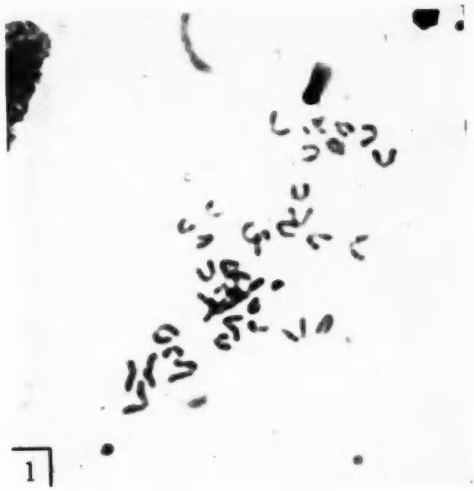


Fig. 1. Photomicrograph of a metaphase of tumour as.7, 5 t.g. with 40 chromosomes. ×1800.

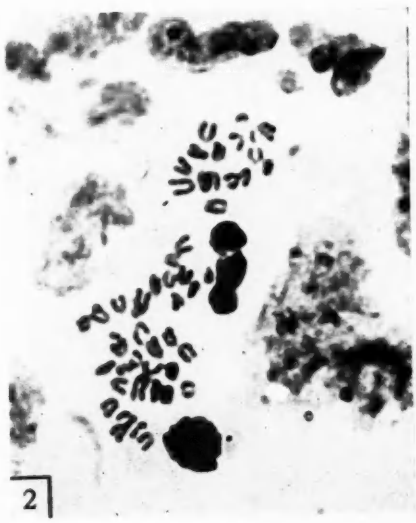


Fig. 2. Photomicrograph of a metaphase of variant as.7/1SW, 3 t.g. with 48 chromosomes. ×1800.

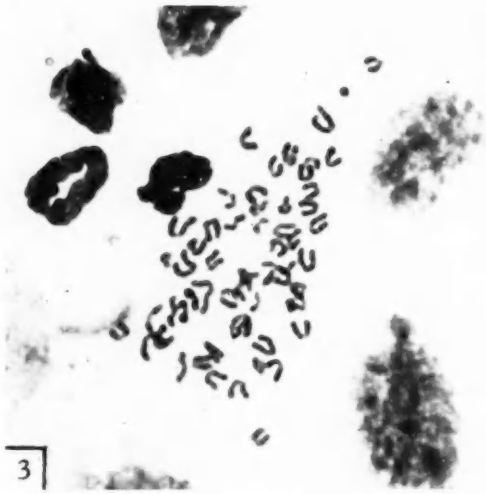
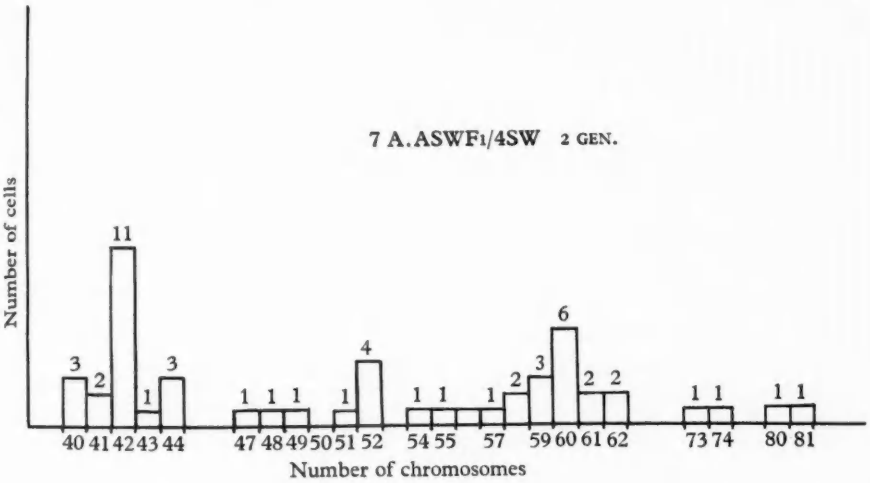
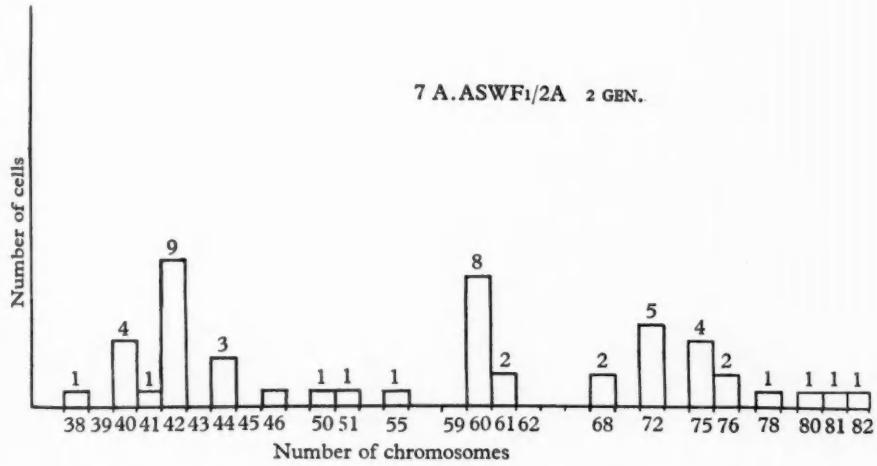


Fig. 3. Photomicrograph of a metaphase of variant as.7/48W. 3 t.g. with 60 chromosomes. ×1800.

Fig. 4. Photomicrograph of a metaphase of variant as.7/2A, 2 t.g. with 72 chromosomes. ×2500.



Text-fig. 4. Distribution of the chromosome number of the variant 4SW, 2 t.g., isolated after TEM treatment from tumour as.7, based on fifty metaphases.



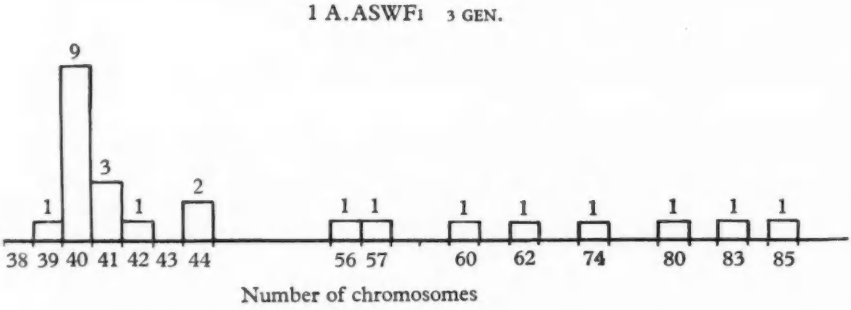
Text-fig. 5. Chromosome number distribution of variant 2A, second generation isolated from tumour as.7, after TEM treatment. Fifty metaphases were examined.

generation in A strain mice showed that it had almost equal numbers of cells with the hyperdiploid number (modal number 42) and with the triploid number of chromosomes. The chromosome number distribution for this variant based on fifty metaphases is shown in Text-fig. 5. It also contained a high proportion of cells at the hypotetraploid number (Plate II, Fig. 4). In accordance with the findings of Hauschka & Levan (1953), this would explain the incomplete specificity of this variant for the A strain.

As far as the hyperdiploid modal number of variants as.7/4SW and as.7/2A (both of which were isolated after TEM treatment) is concerned, there appears to have been a shift from 40 chromosomes in the original tumour to 42 chromosomes in the variants. Both these variants, compared with the original tumour and the spontaneous variant, have a larger proportion of cells around triploid and hypotetraploid numbers. This suggests that TEM induces polyploidy. This action of TEM has been demonstrated by Yerganian (1956) for the chromosomes of the Chinese hamster.

Tumour as.1

The cytology of this tumour at its third transfer generation in hybrid mice was examined. Only twenty-five metaphases were studied and chromosome counts made. Text-fig. 6 shows the chromosome number distribution for this variant. It has a modal number of diploid cells with occasional cells around the triploid and tetraploid numbers. No marker chromosomes again could be detected for this tumour and detailed idiograms were not prepared.

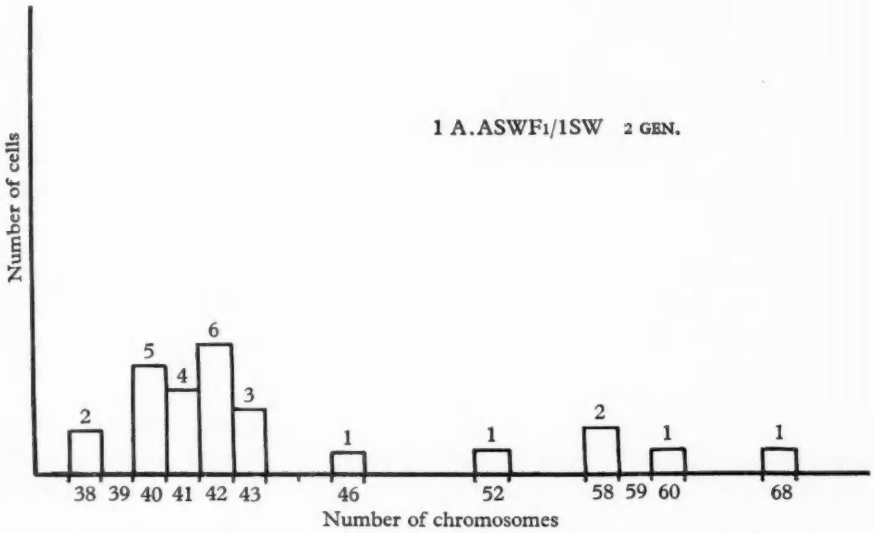


Text-fig. 6. Chromosome number distribution of tumour as.1 at its third generation, based on twenty-five metaphases.

Variant as.1/1SW

This variant was isolated in an A.SW mouse from tumour as.1 at its first transfer generation. The variant was specific for A.SW strain. Again twenty-five metaphases were examined after the variant had been passaged for two transfer generations in A.SW mice. The chromosome number distribution for this variant

is given in Text-fig. 7. Compared with the original tumour, this variant has an increased number of cells with hyperdiploid number of chromosomes, although there was not a complete shift towards hyperdiploidy—chromosome counts ranged from 38 to 43. A few cells with chromosome complements around the triploid number were present.



Text-fig. 7. Distribution of chromosome number in twenty-five metaphases of variant 1SW, 2 t.g., of tumour as.1, which originated spontaneously.

DISCUSSION

The aim of the present study was mainly to investigate the feasibility of using histocompatibility markers in isogenic strains of mice to study the effects of various mutagens on mammalian cells. Although the preliminary nature of the experiments need hardly be stressed, they indicate the advantages and disadvantages involved in using such a method.

Hybrid tumours induced in various combinations of isogenic strains of mice have been used to detect variants compatible with one of the parental strains (Mitchison, 1956; Klein, Klein & Révész, 1957; Klein & Klein, 1958, 1959). It has been shown by these workers that it is possible to obtain variants from heterozygous tumours selectively compatible with one of the parental strains. The results were compatible with the hypothesis that the variants were the result of a genetic change. While no direct evidence can be obtained in support of the occurrence of a mutation in somatic cells, a number of criteria can be used in support of it. If it is assumed that a mutation has taken place, response to mutagenic treatment would support the assumption.

One of the interesting features of variant production by the hybrid tumours is the asymmetry of response towards the two parental strains. With tumours of any hybrid combination one parental strain is always favoured for variant production (Klein & Klein, 1958, 1959). In the present investigation this asymmetry of response is also very marked. Tumour as.7 gave variants only in A.SW mice in the range of 33·3–41·7 % in a number of different experiments. This range is higher than that of MSWB tumour of Klein, Klein & Révész (1957), which gave variants in 15–25 % of A.SW mice (Klein & Klein, 1958). In contrast to this tumour, another tumour, as.14, gave variants specifically compatible with both the parental strains. Klein, Klein & Révész (1957) and Klein & Klein (1958) failed to get variants towards this parental strain with hybrid tumours of the cross A × A.SW. It thus appears that a hybrid tumour of the cross A × A.SW can give selectively compatible variants towards both the parental strains, although it still showed a preference for the A.SW strain.

As has been pointed out by Klein & Klein (1958), it is interesting to note that tumours induced in hybrids of the same inbred strains by the same dose of carcinogen in the same tissue should show such large differences in their transplantation behaviour. Most of the tumours were very specific while some gave variants towards one of the parental strains. Three of the tumours became non-specific after a number of passages in hybrid mice. As two of these tumours had given specific variants towards the A.SW strain before becoming non-specific, it may be postulated that the production of specific variants was a preliminary step to the tumours becoming non-specific and that the two phenomena are comparable. However, a subline of tumour as.7 which gave specific variants towards the A.SW strain and became non-specific in its seventh transfer generation was derived from 10^2 cells at its fifth transfer generation. This subline has remained specific to date, although still producing variants towards A.SW strain, and has been carried to its tenth transfer generation in hybrid mice. It is noteworthy that different sublines of the same tumour can change differently in their transplantation behaviour.

To estimate the mutagenic effectivity of mutagens, chemical or physical, it is essential to calculate the viability of the cells after treatment with the mutagenic agent. This is also necessary in order to establish a dose high enough to produce genetical effects without killing a large proportion of the cells. Staining of the tumour cell suspensions with 1 % Nigrosin in Tyrode solution was used to distinguish between alive and dead tumour cells (Kaltenbach, Kaltenbach & Lyons, 1958). It was found that the percentage of unstained cells to stained cells was the same after treatment with TEM or X-rays as before treatment. This shows that killing of the tumour cells by treatment with X-rays or TEM could not be detected by the staining technique. Vinegar (1956) using microfluorescence cell-viability test with acridine orange to differentiate dead and live cells in ascites tumours found that the test was successful in detecting death due to acid, base, ethanol, diphenylamine diazomium bromide, aerobic autolysis and ultrasonic radiation. He failed to detect tumour cell injury caused by X-rays, a nitrogen mustard and

bromide ion. Kaltenbach *et al.* (1958) were also able to increase the proportion of stained cells by treating ascites tumours with a hypotonic solution. It would appear, therefore, that staining of tumour cell suspensions can detect death due to cytotoxic agents. However, damage caused by X-rays and TEM cannot be detected, as the damage in this case and in the case of nitrogen mustard and bromide ions could be nuclear. The cell would thus need to divide before the damage could be detected. Viability of tumour cells after treatment with X-rays, TEM and possibly other mutagens could, therefore, only be detected by titration of the untreated and treated cell suspensions in groups of mice.

Viability of the tumour cells after treatment with TEM or X-rays was estimated by titrating the untreated and treated cell suspensions in groups of mice and on the chorioallantoic membranes (CAM) of groups of developing chick embryos. The TD_{50} values obtained by titrating cell suspensions on groups of mice or on the CAMs of groups of chick embryos gave comparable results although titration in mice gave more consistent results.

Titration experiments with hybrid sarcomas showed that approximately 10^2 untreated cells (the cell count being based on unstained, presumably viable cells) were needed to produce a tumour. This is actually an underestimate as the above results were based on unstained cells. It would appear that single cells fail to produce solid carcinomas or sarcomas. However, ascites tumours and leukemias have been conclusively proven to be transplantable with a single isolated cell. This has been demonstrated for the Yoshida rat ascites sarcoma (Ishibashi, 1950) and for the Ehrlich and Krebs 2 ascites carcinoma (Hauschka, 1953 *a*, *b*; Hauschka & Levan, 1958).

Although a single cell may be unable to produce a sarcoma in compatible mice, it does not follow that a single variant cell is unable to produce a variant when tested in the parental strains. In this case, the single compatible cell and the large number of incompatible cells undergo a number of divisions before the homograft reaction of the host selectively destroys the incompatible cells. It is possible, therefore, that a single variant cell which would undergo divisions and form a clone could then give rise to a variant tumour. This is substantiated to some extent by the experiments of Klein & Klein (1956 *a*). They obtained takes in mice where 4 compatible cells were mixed in 10^7 incompatible cells.

The percentage of variants produced is considerably decreased when the hybrid tumours are tested in pre-immunized parental mice. In the present investigation, tumour as.7 gave variants in only 7·7 % of pre-immunized A.SW mice compared with 35–40 % in untreated mice. Klein & Klein (1958) obtained only 2 % variants in pre-immunized A.SW mice with the MSWB tumour which gave 20–25 % variants in untreated mice. It has been suggested that the difference is due to the fact that in pre-immunized mice variant production is only due to the selection of pre-existing variant cells while in untreated mice a number of variant cells may be produced during the division of the incompatible cells. As the incompatible cells, when inoculated in pre-immunized mice, undergo no or very limited division before they are destroyed by the homograft reaction, it is possible that the lower

percentage of variants produced may be due to the requirement of a larger number of variant cells to produce a tumour. This would have to be checked by titrating mixtures of compatible and incompatible cells in untreated and pre-immunized or hyper-immunized mice.

Although the results obtained did not give quantitative estimates, it was established that the hybrid tumours do respond to mutagenic treatment. Treatment with TEM or X-rays of tumour as.7 increased the percentage of variants per treated cells towards the A.SW strain. With the exception of one experiment, no variants were produced towards the A strain after treatment. Only one of four variants isolated in the parental strain A after treatment with TEM was specific for this strain, while two others did not show complete specificity for the A strain. One of these variants grew in both A and A.SW mice, but failed to grow in mice of foreign genotypes. Variants isolated in A.SW mice after treatment with TEM showed greater lack of specificity for A.SW mice than the variants isolated spontaneously from the same tumour. It appears, therefore, that TEM treatment induces a certain proportion of non-specific variants. Cytological findings give some support to this.

The cytological analysis of the original tumours and variants isolated without and with treatment by TEM gave interesting results. It was found that the variants isolated without any treatment had a different modal number of chromosomes. While the original tumour line had a mode at approximately 40 chromosomes, the variants usually were hyperdiploid. The variants isolated after treatment with TEM gave different karyotypes compared with the untreated variant. While the original tumour and untreated variants had only a small proportion of triploid cells and very few hypo- and hyper-tetraploid cells, variants resulting after treatment with TEM had a large proportion of cells around the triploid and tetraploid number. A difference was also noted between the variants isolated after TEM treatment in A and A.SW strains. The A.SW variant had few cells around the tetraploid number while the A strain variant had a comparatively larger number of hypotetraploid cells. In agreement with the findings of Hauschka & Levan (1953), who found a correlation between tumour specificity and polyploidy of the cells, the above cytological findings would explain the incomplete specificity of the variants resulting after TEM treatment.

These results are in agreement with those of Bayreuther & Klein (1958) although the cytology of our variants was not examined in as much detail as in their work. They found that each independently isolated variant of MSWB tumour had a characteristic karyotype which was distinct from the original tumour. While the original tumour had a stemline at the diploid number, the variants were always hyperdiploid.

A definite increase in the frequency of variants produced is noted after treatment with X-rays or TEM. Variant production appears to be always associated with chromosomal derangements. In the case of TEM, the increase in variant frequencies appears to be partly due to its cytological effects. A certain percentage of non-specific variants are produced after TEM treatment due to the presence of larger numbers of polyploid cells.

The nature of the mechanism of variant formation in heterozygous tumours and the question whether these variants are due to genetical or non-genetical modifications, have been discussed by Klein & Klein (1959). From the fact that true-breeding variants are obtained with heterozygous but not with homozygous tumours, they conclude that the mechanism of variant formations is probably genetical or nuclear in origin.

At the genetic level, possible mechanisms include somatic crossing over, point mutations, deletions, loss of a chromosome or larger chromosomal changes, and transduction. These have been dealt with by Klein & Klein (1958, 1959) and need only be mentioned. Transduction, involving the change of genetic material between the host and tumour cells, appears to be unlikely as transduction has not yet been demonstrated for mammalian cells.

The use of heterozygous tumour cells is a prerequisite to studying mutations with histocompatibility markers as the probability of a double mutation occurring at the same locus is infinitesimally small. Hence, the possibility of somatic crossing over in heterozygous tumour cells cannot be ruled out.

The question whether variant formation could be due to point mutations at the histocompatibility locus is a difficult one. One objection to point mutation is that most of the isogenic resistant strains differ with regard to two or three antigenic components and it is unlikely that single point mutations could alter a number of antigenic components. However, Hoecker (1956) has suggested that a single genetical change may induce a multiple change of serological specificities.

The fact that all the variants have a hyperdiploid number of chromosomes suggests that duplication of some genetic material may play a role in variant formation. Sachs & Gallily (1956) have shown that not all the chromosomes have to be duplicated for a tumour to become non-specific. It may be postulated that duplication of a chromosome containing one H-2 allele may make a variant non-specific for that allele, whatever may be the mechanism of non-specificity in relation to chromosome duplication. This variant would then be specific for the other H-2 allele. Thus, duplication of the chromosome carrying H-2a would give a cell with the genctype H-2a/H-2a/H-2s, which would only be specific for the H-2s allele.

While variant formation in hybrid sarcomas appears usually to be associated with cytological changes, Hellström found variants among hybrid lymphomas where no cytological changes could be detected (Klein & Klein, 1959). This suggests that tumours other than sarcomas with more stable cytological constitutions may be more suitable for mutation studies with isogenic strains of mice.

SUMMARY

The feasibility of using heterozygous tumours induced in hybrids between Snell's isogenic resistant (IR) strains of mice for mutation studies was examined. The system has been described by Mitchison (1956) and Klein, Klein & Révész (1957).

Fourteen hybrid sarcomas (A × A.SW F_1; H-2 genotype a/s) were tested in mice of the parental strains. Seven were specific for the original F_1 hybrid genotype and failed to give any variants. Four tumours gave variants that were compatible with one of the parental strains. The variants grew progressively in all mice of the parental strain, even in pre-immunized mice, and failed to grow in mice of foreign genotypes. Of these, one tumour, as.14 gave true variants towards both the parental strains. One tumour, as.7, gave variants regularly in 35·5% of the A.SW mice but only in 7·7% of pre-immunized A.SW mice. It failed to give variants towards the other parental strain.

The effect of triethylenemelamine (TEM) and X-ray treatment on the rate of variant production in hybrid tumours was studied. The effect of the treatment on the viability of tumour cells was first examined by titrating tumour cell suspensions on groups of mice or on the CAMs of groups of developing chick embryos. The TD_{50} values (50% end-points) were calculated. Comparable results were obtained by titrating on mice and on the CAM, although titration on mice gave more consistent results. Approximately 10^2 viable, untreated cells were needed to produce a tumour in mice or on the CAM.

Tumour as.7 was tested in the parental strains after treatment with TEM or X-rays. Using comparable cell doses in the untreated controls and treated series, the percentage of variants produced in one of the parental strains (A.SW) was significantly increased after treatment with TEM or X-rays. With the exception of one experiment, no variants were produced in the other parental strain. In one experiment, four variants were produced towards the A strain after treatment with TEM, three of which were tested: only one was found to be specific for the A strain. Three of the A.SW variants, originating after treatment with TEM, were tested: two were specific while one showed incomplete specificity for the A.SW strain. Treatment of specific tumours with TEM or X-rays did not give variants towards either of the parental strains.

Two of the hybrid tumours and their derived variants were examined cytologically. Variants originating spontaneously and after treatment with TEM were examined. While most of the original tumours had chromosome numbers with an exactly diploid modal number, the spontaneous and TEM-treated variants had modal numbers which were hyperdiploid. The TEM-treated variants also had a larger proportion of cells which were triploid or hypotetraploid. This would explain the incomplete specificity of the variants isolated after treatment with TEM.

While it appears possible to use heterozygous tumours for isolating genetical variants specifically compatible with one of the parental strains, the actual nature of the mechanism of variant production remains dubious.

I am grateful to Dr C. Auerbach, F.R.S., for her supervision and encouragement during the course of this study. My sincere thanks are due to Dr N. A. Mitchison for teaching me many of the techniques and for valuable advice.

I am also grateful to Professor C. H. Waddington, F.R.S., for laboratory facilities, and to

Professor G. Klein, Karolinska Institute, Stockholm, for supplying the mice. I would like to thank Dr Sellers for helping with the cytological photographs.

This work was done under a grant from the Government of the Federation of Malaya which is gratefully acknowledged.

REFERENCES

ALLEN, S. L. (1955). H-2f, a tenth allele at the histocompatibility-2 locus as determined by tumour transplantation. *Cancer Res.* **15**, 315–319.

BAYREUTHER, K. & KLEIN, E. (1958). Cytogenetic, serologic and transplantation studies on a heterozygous tumour and its derived sublines. *J. nat. Cancer Inst.* **21**, 885–923.

BRITISH EMPIRE CANCER CAMPAIGN (1951). *Annual Report*, p. 58.

HAUSCHKA, T. S. (1953 *a*). Cell population studies on mouse ascites tumours. *Trans. N.Y. Acad. Sci.* **16**, 64–73.

HAUSCHKA, T. S. (1953 *b*). Methods of conditioning the graft in tumour transplantation. *J. nat. Cancer Inst.* **14**, 723–739.

HAUSCHKA, T. S. & LEVAN, A. (1953). Inverse relationship between chromosome ploidy and host specificity of sixteen transplantable tumours. *Exp. Cell Res.* **4**, 457–467.

HAUSCHKA, T. S. & LEVAN, A. (1958). Cytologic and functional characterisation of single cell clones isolated from the Krebs-2 and Ehrlich ascites tumours. *J. nat. Cancer Inst.* **21**, 77–135.

HOECKER, G. (1956). Genetic mechanisms in tissue transplantation in the mouse. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 355–362.

ISHIBASHI, K. (1950). Studies on the number of cells necessary for the transplantation of Yoshida Sarcoma. *Gann*, **41**, 1–24.

KALTENBACH, J. P., KALTENBACH, M. H. & LYONS, W. B. (1958). Nigrosin as a dye for differentiating live and dead cells. *Exp. Cell Res.* **15**, 112–117.

KLEIN, E., KLEIN, G. & RÉVÉSZ, L. (1957). Permanent modification (mutation?) of a histocompatibility gene in a heterozygous tumor. *J. nat. Cancer Inst.* **19**, 95–114.

KLEIN, G. & KLEIN, E. (1956 *a*). Detection of an allelic difference at a single gene locus in a small fraction of a large tumor cell population. *Nature, Lond.*, **178**, 1389–1391.

KLEIN, G. & KLEIN, E. (1956 *b*). Mechanism of induced change in transplantation specificity of a mouse tumor passed through hybrid hosts. *Transplant. Bull.* **3**, 136–142.

KLEIN, G. & KLEIN, E. (1958). Histocompatibility changes in tumours. *J. cell. comp. Physiol.* **52**, Suppl. 1, 125–168.

KLEIN, G. & KLEIN, E. (1959). Studies of histocompatibility mutations in isogenic strains of mice. In *Symposium on Biological Problems of Grafting*, Liege, April 1959.

LEDERBERG, J. (1956). Prospects for a genetics of somatic and tumor cells. *Ann. N.Y. Acad. Sci.* **63**, 662–665.

MITCHISON, N. A. (1956). Antigens of heterozygous tumours as material for the study of cell heredity. *Proc. roy. Phsy. Soc.* **25**, 45–48.

MURPHY, J. B. (1912). Transplantability of malignant tumors to the embryo of a foreign species. *J. Amer. med. Ass.* **59**, 874–875.

PUCK, T. T. (1957). The genetics of somatic mammalian cells. *Advanc. biol. med. Phys.* **5**, 75–101.

PUCK, T. T. (1958 *a*). Action of radiation on mammalian cells. III: Relationship between reproductive death and induction of chromosome anomalies by X-irradiation of euploid and human cells *in vitro*. *Proc. nat. Acad. Sci., Wash.*, **44**, 772–780.

PUCK, T. T. (1958 *b*). Genetics of somatic mammalian cells. *J. cell. comp. Physiol.* **52**, Suppl. 1. 287–311.

PUCK, T. T. & CIECIURA, S. J. (1958). Studies on the virus carrier state in mammalian cells. In *Symposium on Latency and Masking in Viral and Ricketssial Infections*, Burgess Publishing Co., Minneapolis, pp. 74–79.

PUCK, T. T., CIECIURA, S. J. & FISHER, H. W. (1957). Clonal growth *in vitro* of human cells with fibroblastic morphology. Comparison of growth and genetic characteristics of single epitheliod and fibroblast-like cells from a variety of human organs. *J. exp. Med.* **106**, 145–147.

PUCK, T. T. & FISHER, H. W. (1956). Genetics of somatic mammalian cells. I: Demonstration of the existence of mutants with different growth requirements in a human cancer cell strain (He La). *J. exp. Med.* **104**, 427–434.

PUCK, T. T. & MARCUS, P. I. (1955). A rapid method for viable cell titration and clone production with He La cells in tissue culture. The use of X-irradiated cells to supply conditioning factors. *Proc. nat. Acad. Sci., Wash.*, **41**, 432–437.

PUCK, T. T., MARCUS, P. I. & CIECIURA, S. J. (1956). Clonal growth of mammalian cells *in vitro*. Growth characteristics of clones from single He La cells with and without a 'feeder' layer. *J. exp. Med.* **103**, 273–284.

REED, L. J. & MEUNCH, H. (1938). A simple method of estimating fifty per cent end points. *Amer. J. Hygiene*, **27**, 493–497.

SACHS, L. & GALLILY, R. (1956). The chromosomes and transplantability of tumours. II: Chromosome duplication and the loss of strain specificity. *J. nat. Cancer Inst.* **16**, 803–840.

SNELL, G. D. (1948). Methods for the study of histocompatibility genes. *J. Genet.* **49**, 87–108.

SNELL, G. D. (1953). Analysis of the histocompatibility-2 locus in the mouse. *J. nat. Cancer Inst.* **14**, 457–458.

SNELL, G. D. (1955). Isogenic resistant (IR) lines of mice. *Transplant. Bull.* **2**, 6–8.

VINEGAR, R. (1956). Metachromatic differential fluorochroming of living and dead ascites cells with Acridine Orange. *Cancer Res.* **16**, 900–906.

YERGANIAN, G. (1956). Action of triethylene melamine on tumour chromosomes of the Chinese hamster, *Cricetulus griseus*. *Proc. int. Symp. Genet. Cytologia Suppl.* 206–209.

Genet. Res., Camb. (1961), **2**, *pp.* 333–345
With 2 text figures
Printed in Great Britain

Attachment of wild-type *F* factor to a specific chromosomal region in a variant strain of *Escherichia coli* K12 : the phenomenon of episomic alternation

By ALAN RICHTER*

Department of Genetics, University of Wisconsin, Madison, Wisconsin

(*Received* 21 *October* 1960)

INTRODUCTION

Sex in *Escherichia coli* is determined by a factor called *F*. Those individuals which possess the *F* factor have both donor and recipient potentiality with regard to the conjugal act. Those individuals which lack the *F* factor have recipient potentiality only. The presence of *F* thus determines donor potentiality. We shall refer to individuals which are potential donors of genetic material in conjugation as males and to the others as females or *F* −.

There are two kinds of males depending on the manner of presence of the *F* factor. *F* + males possess the *F* factor in the *infectious* state characterized by two properties. First, the factor is readily transmissible to females upon cell contact as a result of which the females are converted to *F* + males (Lederberg, Cavalli & Lederberg, 1952). Second, in the infectious state, the factor is susceptible to the disinfecting action of the dye acridine orange (Hirota, 1960).

The *F* factor of *Hfr* males is neither contagious (Cavalli, Lederberg & Lederberg, 1953; Hayes, 1953) nor disinfectible (Hirota, 1960). In fact, it has been shown in two cases tested to be located on the chromosome (Richter, 1957*a*, 1958). So, as the presence or absence of the factor distinguishes males and females, it is very likely its position in the cell which distinguishes the two kinds of males. This is summarized in Table 1.

Table 1. *Determination of mating type in* Escherichia coli

Mating type	Position of the *F* factor
Hfr ♂	chromosomal
F + ♂	extrachromosomal
♀	absent

Hfr is the functional male state. *F* + males are probably incapable of acting as donors except after a genetic change to *Hfr*. The evidence for this view is the finding that donor ability fluctuates among *F* + cultures to a greater degree than can be accounted for by sampling error (Jacob & Wollman, 1956). The fertility of

* Present address: Wistar Institute of Anatomy and Biology, Philadelphia 4, Pennsylvania, U.S.A.

an $F+$ strain may thus be considered to depend on its rate of change to *Hfr*. For example, some $F+$ strains are male-sterile (i.e. they do not yield genetic recombinants in crosses with $F-$); some change to *Hfr* at rates about 10^{-6} to 10^{-5}; certain mutable strains, such as the strain to be discussed in this paper, change at much higher rates. We shall call those $F+$ males *standard* which change to *Hfr* at the same rate as the type strain K12, i.e. 10^{-6} to 10^{-5}.

Standard $F+$ strains change at a low rate to various kinds of *Hfr* in which the *F* factor is presumably fixed to different positions on the chromosome (Jacob & Wollman, 1958*a*). The variant $F+$ strain to be described changes at a high rate to a specific kind of *Hfr*.

This paper discusses the genetic structure and behaviour of a strain of *Escherichia coli* which reversibly alternates between a specific *Hfr* state and the $F+$ state.

MATERIALS AND METHODS

Method of crossing

Most crosses were performed in liquid medium prior to plating. The standard cross procedure was as follows. Isolated single colonies of the parental strains were inoculated to tubes containing 10 ml. of 'Penassay' broth (Difco) and incubated without aeration for 15–18 hours at 37°C. After incubation the two cultures were mixed in the following amounts: 1 ml. each parent and 3 ml. fresh Penassay broth. The mixture was incubated without aeration for 2 or 3 hours at 37°C. It was then either plated on the appropriate selective medium or first washed before plating. Washing was carried out by centrifuging the mixed culture, decanting the supernatant, washing the pellet with 2 ml. water, and resuspending at the original concentration of cells in water or 1% saline. Cross mixtures were plated at dilutions which would yield 50 to 100 recombinant clones per plate, except in the case of crosses plated on EMB medium where it was found necessary to plate more dilute suspensions. (In this way it was possible to avoid selective pressures against certain recombinants on EMB medium. The region of chromosome conferring selective disadvantage is linked to the locus determining the threonine(*T*) requirement of the T^- parent.) The cross plates were incubated at 37°C. for 30 to 40 hours at which time the recombinant colonies were picked and streaked on EMB medium. Isolated single colonies from these streak plates were used for the scoring of markers. Replica plating with cotton velvet was employed for scoring.

Some crosses were made directly on selective agar medium by plating the parental suspensions together on the same plates.

Use of media

Difco Penassay broth was used for the growing of cultures. *EMB complete medium* supplemented with 1% sugar, or 1·5% in the case of maltose and 2% in the case of lactose, was used for scoring fermentation markers. EMB + sugar was also used as a selective medium for fermenters in conjunction with selection for

resistance to streptomycin or phage. Phage was added to the cross plates when the bacteria were plated, while streptomycin was incorporated into the medium at a concentration of 0·2 mg per ml. *EMS minimal indicator medium* was employed for certain crosses in order readily to ascertain the proportion of fermenters among the progeny. Most of the crosses, however, were plated on *Davis minimal medium* (DO medium) containing 0·5 % glucose. Supplements were added to minimal cross plates in these concentrations (gamma per ml.): leucine, threonine and arginine, 40; methionine, proline and histidine, 20; thiamine, 0·25. The same supplements were used at half-concentration for scoring nutritional markers. All these media are described by Lederberg (1950). In addition, *EM Galactose* is EMS medium minus succinate with 1 % galactose added. *EM Lactose* and *EM Maltose* are similar media, but with 2 % and 1·5 % concentration of sugar, respectively.

Tests for mating type

Segregants from crosses were tested for mating type by spot tests against female tester strains on appropriate selective media. For these tests the EM sugar media were particularly useful since the sugar supplement is the sole carbon source. This feature enabled selection for fermentation markers. Primary crosses were usually set up so that one marker was left available for progeny tests. For example, from the cross of $M^- Gal_2^- \times T^- L^- Gal_2^-$ on minimal medium, all progeny are $M^+ Gal_2^-$ and can therefore be tested against a female $M^- Gal^+$ tester on EM Galactose. The method of testing is as follows. Purified recombinants are inoculated to 1-ml. broths and grown up. A broth culture of the female tester is streaked across an EM Galactose plate. When the streak has dried, the recombinants are spotted on top of the streak by means of a loop. Control spots of the cultures to be typed may be placed on the same plate of EM Galactose. The plates are incubated and read at 24 and 48 hours. The occurrence of galactose-positive papillae is taken as an indication that recombination has occurred. Other useful spot tests are performed on EM sugar media supplemented with streptomycin. Direct spotting of S^s cultures against female S^r testers may be employed.

F *transmission*

Tests for the transmission of the *F* factor were performed according to methods described by Cavalli *et al.* (1953).

Markers and strains

A list of genetic markers is found in Table 2 and a list of bacterial strains in Table 3. All strains are derivatives of strain K12.

Efficiency of recombination

Efficiency of recombination (E.R.) is the reciprocal of the number of cells of a standard parental mixture required to give one recombinant, as determined from platings which yield 50–100 recombinants. Standard conditions have been defined above.

Table 2. *List of genetic markers*

Fermentation of sugars	Nutritional requirements	Drug resistance
+ = ferments,	+ = independent,	s = sensitive, r = resistant
− = does not	− = dependent	*S* streptomycin
Gal galactose	*Th* thiamine (B_1)	
Mal maltose	*M* methionine	Episomes
Xyl xylose	*T* threonine	♂ = present, ♀ = absent
	L leucine	*F* sex factor

Table 3. *List of bacterial strains, their designations and genotypes*

Designation	Genotype*
K12	$F+$ prototroph (λ)
W1321	♀ $M^- S^r Gal_4^-$
W1578	♀ $M^- Lac^- Gal_4^-$
W2580	♀ $T^- L^- Th^- Gal_2^- Xyl^-$ (λ)
W2660	♀ $T^- L^- Th^- S^r$
W2817	$F+ T^- L^- Th^- S^r$
W2924	♂$_3$ $T^- L^- Th^- S^r Mal_5^-$
W3062	♂$_3$ prototroph $Mal_5^- Gal_2^-$
W3135	♀ M^-
W3161	♀ prototroph $Gal_7^- Xyl^- S^r$
W3191	♀ $T^- L^- Th^- S^r Xyl^-$
W3234	♂$_3$ $M^- Gal_2^- Mal_5^-$ (λ)

* The genotype lists only the pertinent markers.

EXPERIMENTAL

The strain in question, ♂$_3$, was selected as a maltose-nonfermenting mutant (W2924) after ultra-violet irradiation of an $F+$ culture (W2817). The maltose marker was labelled Mal_5. Prototrophic and M^- derivatives of W2924 were obtained by crossing. As these always showed the same behaviour as the original mutant, they will also be referred to as ♂$_3$.

In early crosses ♂$_3$ behaved unusually in that (1) it was about 10,000 times as fertile as its $F+$ parent strain when the M^+ locus was selected, and (2) it was unstable, giving rise to a less fertile variant which was infectious for the *F* factor.

It was later found that the less fertile variant could in turn give rise to the more fertile variant and consequently the two variants will be referred to as the two states of ♂$_3$.

State I is the more fertile state and state II is the less fertile state. The test for state is thus a test for fertility in crosses.

As shown in Table 4, state I is 100 times more fertile than state II when the

Table 4. *Recombination efficiencies for ♂$_3$ × ♀ crosses*

	Recombination Efficiency	
State of ♂$_3$ culture (W3062)	× ♀ M^- (W1321)	× ♀ $T^- L^-$ (W2660)
I	$2{\cdot}2 \times 10^{-2}$	$6{\cdot}8 \times 10^{-6}$
II	$1{\cdot}4 \times 10^{-4}$	$6{\cdot}3 \times 10^{-7}$

Selection was imposed for $M^+ S^r$ and $T^+ L^+ S^r$ recombinants, respectively. The fraction of state I cells in the state I culture was $0{\cdot}87 \pm 0{\cdot}05$.

recovery of M^+S^r recombinants is taken as the test of fertility. Neither state is especially fertile when the yield of $T^+L^+S^r$ recombinants is taken as the measure of fertility. It should be noted that the recombination efficiency for a cross of standard $F+$ × female varies from 10^{-7} to 10^{-6} regardless of the selective markers employed.

Proof that the fertility of state II populations is due to the presence of state I variants

Cultures in state I transmit the gene M in crosses with an efficiency of 10^{-2}. This level of fertility is comparable to that of stable *Hfr* strains. Cultures in state II transmit M with an efficiency of 10^{-4}, a level intermediate between *Hfr* and standard $F+$ fertility. The transmission of the gene M by cultures in state II can be quantitatively accounted for by the presence of state I variants as shown by the following experiment.

δ_3 strain W3062 (prototrophic, S^s) was streaked out and one colony of each fertility level chosen. These were inoculated to tubes of broth. The broth cultures were plated on EMB Galactose (to determine viable cell counts and the proportion of state I cells in each culture) and also combined to yield mixtures ranging from whole state I culture to 1 part state I culture in 500 parts state II culture. These combinations were crossed by the standard procedure with the M^-S^r female strain W1321 and different dilutions plated on minimal medium + streptomycin. From the proportion of state I cells in the state I culture (0·85; all of 69 isolates of the state II culture were state II), the true proportion of state I cells in each mixture was calculated and plotted against the number of recombinants obtained per 10^{-5} ml. of cross mixture. The straight-line graph of Fig. 1 was obtained, indicating that the E.R. previously defined is a linear function of the concentration of donor cells in the range of efficiencies obtained.

The following counts of state I variants in state II cultures have been recorded in various experiments, including that detailed above: 2/80; 0/69; 1/150; 1/100. The expected recombination efficiency of a state II culture containing 1% state I cells was therefore calculated from the slope of the plot. That value, $1{\cdot}8 \times 10^{-4}$, agrees well with the values ordinarily obtained (see Table 4).

Cultures in state II transmit the F factor efficiently as shown in Table 5. State II may therefore be equated with the infectious state of the F factor, that is the $F+$ state. Since there is no evidence that state II is intrinsically any more fertile than the standard $F+$ strain from which δ_3 arose, we may conclude that the distinction between state II and standard $F+$ is the rate of change to state I, i.e. the *Hfr* condition.

Cultures in state I transmit the F factor inefficiently. It is presumed that cells in state I, like cells of stable *Hfr* genotypes, are not infective for the F factor. This has not been proven. Presence of the $F+$ variants in the state I cultures renders such a proof difficult. In addition, it is likely that a lag occurs between the time of fixation of an F factor on the chromosome and the subsequent loss of all infectious F factors from the cell (see Cavalli-Sforza, 1959; Adelberg & Burns, 1960).

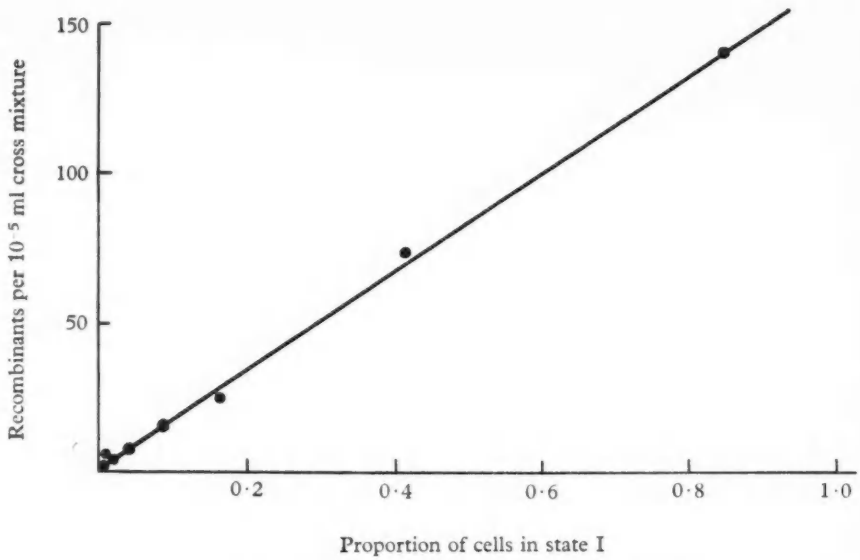


Fig. 1. Yield of recombinants versus cells in state I.

Table 5. F *status of progeny from matings of* ♂$_3$×♀ *and standard* F+ ×♀

Type of donor parent	No. of isolates having mating type: I	II	std. $F+$	F status of recombinants* $F+$	$F-$
State I	98	2	0	10	90
State II	1	99	0	87	13
Standard $F+$	0	0	100	99	1

* Selected for prototrophy. For the first two crosses, W2924 (♂$_3$ $T^-L^-Th^-$) was mated with W1578 (♀ M^-). For the third cross, W2817 ($F+$ $T^-L^-Th^-$) was mated with W1578. For each cross, 100 single colony isolates of the broth culture of the donor parent were tested for mating type versus W3161 on EM Xylose, and 100 purified recombinants were tested versus W3135 on EM Galactose.

The functional male state is therefore inversely correlated with the infectious state of the *F* factor within the ♂$_3$ system just as within *E. coli* K12 in general.

The behaviour of F− *derivatives of* ♂$_3$

It is known that $F+$ strains may give rise to $F-$ variants, i.e. females, which may then be selectively enriched by passage through soft agar (Skaar, Richter & Lederberg, 1957). In addition, one may produce $F-$ variants at will by treatment of growing $F+$ cells with acridine orange (Hirota, 1960). The $F-$ variants obtained in both cases are reconvertible to $F+$ by growth in mixed culture with an $F+$ strain. The reconverted $F+$ display the same low fertility in crosses as the original $F+$ strains. When ♂$_3$ is subjected to either of the above treatments,

one can also obtain $F-$ variants. However, such $F-$, when grown in mixed culture with standard $F+$, are always converted to state II, i.e. to $F+$ possessing high rate of change to state I in distinction from standard $F+$, i.e. $F+$ possessing low rate of change to state I. The $F-$ derivative of $\male_3$ is called $\female_3$ to distinguish it from the $F-$ derivative of standard $F+$ which is called standard $F-$ or female.

Two conclusions may be drawn from the above. First, the presence of F is a necessary condition to the expression of the $\male_3$ and therefore state I phenotype. Second, rate of change is a property of the bacterium which is conserved independently of the presence or absence of F.

Inheritance of the $\male_3$ property

Conjugation in *E. coli* involves the oriented transfer of the bacterial chromosome from the *Hfr* donor to the female recipient. Jacob & Wollman (1958*a*) have shown that the *Hfr* property itself is always the last marker transferred.

When one performs a cross by selecting recombinants which carry a given marker of the *Hfr* parent, markers which are transferred earlier than the selected marker appear in the progeny according to their linkage to the selected marker. Markers transferred later than the selected marker may not appear at all. According to current theory this is so because the transfer of the *Hfr* chromosome is usually incomplete, resulting in zygotes that are incomplete.

Since the *Hfr* property is the last marker to be transferred, it is not possible to ensure a set of complete zygotes for the genetic analysis of the *Hfr* property. One must therefore do the best one can, which is to select recombinants carrying a marker of the *Hfr* parent as near as possible to the *Hfr* marker. Table 6 presents

Table 6. *Linkage of the $\male_3$ character*

Cross	$\male_3$ character of progeny	No. of T^+L^+ recombinants of phenotype: *Th* +	*Th* −	*Mal*$_5$ −	*Mal*$_5$ +	State of $\male_3$ progeny I	II
A. $\male_3$ state I × ♀	$\male_3$	*9*	8	*17*	0	13	4
	Non-$\male_3$*	19	*25*	0	*44*		
B. $\male_3$ state II × ♀	$\male_3$	*5*	3	*8*	0	1	7
	Non-$\male_3$*	1	*46*	0	*47*		

The crosses were W3062 ($\male_3$ $T^+L^+Th^+Mal_5^-$) × W3191 (♀ $T^-L^-Th^-Mal_5^+$). The italic figures represent the parental couplings.

* The non-$\male_3$ progeny include standard $F-$ and standard $F+$.

the data of such an experiment. W3062 was mated in each of its states with a female strain, W3191, and selection was imposed for $T^+L^+S^r$ recombinants. Both crosses demonstrate the tight linkage of the $\male_3$ property and *Mal*$_5$, the mutation which arose simultaneously with $\male_3$.

It is concluded that the δ_3 property is inherited as a single gene difference from standard female.

Now, these rare recombinant progeny which receive the δ_3 property apparently do not arise solely from matings of state I cells with female cells as is true for the M^+ recombinants. This is shown by the fact that the majority of the δ_3 progeny arising from a mating of state II × female are in state II, while the majority of the δ_3 progeny arising from a mating of state I × female are in state I. Since the linkage relation between state and Mal_5 is the same in the two states, the determinants of the two states may be regarded as alleles. The mating phenotypes in question preclude a direct test of allelism.

Although the two crosses agree in respect to linkage to *Mal*, they do not agree in respect to *Th*. The determinant of state II is linked to the marker *Th* (interaction = 77) while the determinant of state I is not (interaction = 1·5). Interaction equals the product of the two parental types divided by the product of the two non-parental types. These and other crosses have shown that the relationships of all other markers are the same in the two states and that these relationships are the same as found in all other strains of K12 (Richter, 1957*b*). It is therefore concluded that the δ_3 property lies between *Th* and *T* in the sequence *MTh* (δ_3-Mal_5)*TL* and that this block of markers is uncoupled in state I. The linkages found in state I are *MTh* and (δ_3-Mal_5)*TL*. The significance of this uncoupling will be discussed later.

Location of the F *factor at the site of the determinant of rate of change*

Since, as shown above, rate of change is a property of the bacterium *per se*, one ought to be able to follow it in crosses independently of the δ_3 character. One can then survey all progeny which have inherited high rate of change and ask how many are also δ_3 in phenotype.

To this end W3234 (δ_3 $M^-Gal_2^-$) in state I was mated with W2580 (♀ $T^-L^-Th^-Gal_2^-$) on minimal (DO) agar. In order to minimize *F* transmission by cell contact, a low multiplicity of male cells was employed. Thus, 0·5 ml. of washed female culture was plated together with 10^{-2} ml. of washed male culture. The prototrophs obtained were necessarily Gal_2^-. Every isolated colony on one cross plate was picked, purified, checked for maltose fermentation and prototrophy, inoculated into a 1-ml. broth, and checked for mating type versus W3135 on EM Galactose. The results are presented in Table 7A(1). Of the thirty-two δ_3 progeny, there were thirty-one in state I and one in state II. Of the fifty non-δ_3, there were six Mal^+ standard $F+$ and forty-four females. Each of the forty-four females was grown in mixed culture with K12 and reisolated. The reisolated clones were then tested for mating type by crosses with W3135 on EM Galactose as before sex conversion. The results are shown in Table 7A(2). All forty-three Mal^+ females were converted to standard $F+$; the single Mal^- female was converted to state II. It must, then, have inherited high rate of change from its δ_3 parent. From the data, one can reconstruct the genotypes of all the progeny with respect to rate of change. This

is done in Table 8A. From the mating of state I × ♀, thirty-three progeny inherited high rate of change, of which thirty-two exhibited the ♂$_3$ phenotype before sex conversion. Thus, almost all progeny which inherit high rate of change are ♂$_3$. But, as we concluded earlier, the ♂$_3$ phenotype depends on the presence of the *F*

Table 7. *Assortment of* Mal_5 *and mating type before and after sex conversion in crosses of* ♂$_3$ × ♀

		No. of $T^+L^+Th^+$ recombinants of mating type:							
		(1) before sex conversion of all female progeny				(2) after sex conversion of all female progeny			
		♂$_3$		Non-♂$_3$		♂$_3$		Non-♂$_3$	
		I	II	std. *F*+	♀*	I	II	std. *F*+	♀
A. State I × ♀	Mal_5 −	31	1	0	1	31	2	0	0
	+	0	0	6	43	0	0	49	0
B. State II × ♀	Mal_5 −	1	39	0	4	1	43	0	0
	+	0	0	22	1	0	0	23	0

* These include ♀$_3$. The crosses were W3234 (♂$_3$ $M^-Gal_2^-Mal_5^-$) × W2580 (♀ $T^-L^-Th^-Gal_2^-$).

factor. Therefore, F *is almost always inherited whenever high rate of change is inherited.* Now, in the case of state I × ♀, *F* is inherited by a minority of the wild-type progeny, i.e. progeny standard for rate of change (Table 8A as well as Table 5). Therefore, in state I the *F* factor must be located at the site of the determinant of rate of change.

Table 8. *Inheritance of* F *and rate of change in crosses of* ♂$_3$ × ♀ *(from data of Table 7)*

		No. of $T^+L^+Th^+$ recombinants of stated genotype		
		Total	♂	♀
A. State I × ♀	high rate of change	33	32	1
	standard rate of change	49	6	43
B. State II × ♀	high rate of change	44	40	4
	standard rate of change	23	22	1

The symbols ♂ and ♀ are used to denote the presence and absence of *F*, respectively. Rate of change is determined after sex conversion as described in the text.

A similar cross was performed between W3234 in state II and W2580. The results are given in Tables 7B and 8B. In this case, *F* is inherited by a majority of the progeny whether they have inherited high rate of change or not. The cross yields no information.

The determinant of rate of change is given the locus symbol Hfr_3.

DISCUSSION

These results cannot be discussed apart from the *episome* concept introduced by Jacob and Wollman (1958*b*). According to this view there are hereditary elements which can multiply either integrated with the chromosome, like prophage, or autonomously of the chromosome, like vegetative phage. These elements may be present in the cell in either of the above two states (integrated or autonomous), or absent from the cell entirely, as in a bacterium sensitive to phage.

The present work establishes the episomal nature of the *F* factor.

The *F* factor may be present (males) or absent (females) from the cell and, when present, may be either integrated (*Hfr* male) or autonomous (*F*+ male).

In the δ_3 strain there is an accelerated variation between the *F*+ state and the

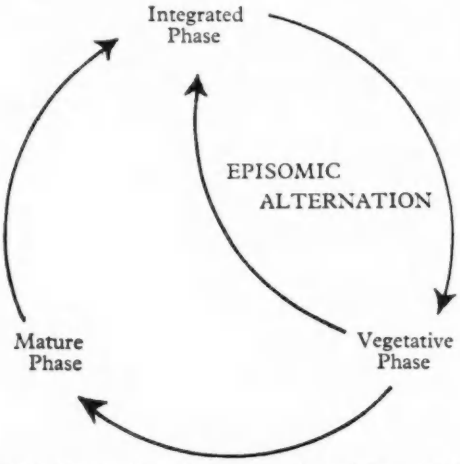


Fig. 2. Diagram of the lysogenic cycle showing the relation of episomic alternation.

Hfr state, involving always the same chromosomal site. This variation is the reflection of the *dance* of the *F* factor. In state II the *F* factor multiplies in the autonomous state (*F*+ →*F*+). The attachment of an *F* factor to the Hfr_3 locus initiates state I (*F*+ →*Hfr*). In state I the *F* factor multiplies in the integrated state (*Hfr*→*Hfr*). The detachment of the *F* factor from the Hfr_3 locus initiates state II (*Hfr*→*F*+) and the cycle of movements is completed. The back-and-forth shift of an episome between its integrated state of multiplication at a single locus and its autonomous state of multiplication is termed *episomic alternation*. Episomic alternation may be viewed in relation to the lysogenic cycle as shown in Fig. 2. The reader should refer to the article on lysogeny by Lwoff (1953).

The attachment of the *F* factor to the chromosome has as a consequence the disruption of linkages known to be present in the *F*+ state. In the δ_3 system, the Th–Mal_5 linkage found in state II is uncoupled in state I. This could be interpreted in one of three ways. (1) The change from *F*+ to *Hfr* involves the breaking of the chromosome. The break is perpetuated in vegetative divisions of the *Hfr*

bacteria. (2) *F* attaches to the chromosome at the change but the chromosome does not become broken until mating. (3) The change from *F*+ to *Hfr* involves the insertion of the episome into the chromosome. If the episome had an end which was unable to form an internucleotide linkage, the gap, so to speak, would be built into the structure of the episome-bearing chromosome. This gap could be in a single strand of deoxyribonucleic acid, rather than a gap in the duplex.

Episomic alternation and local change of state

The analysis of mutable loci in maize has shown that these loci are the sites of accessory elements. These are called controlling elements because they tend to suppress gene action at the loci where they reside (McClintock, 1956). A mutable allele, such as the variegated pericarp allele at the *P* locus, is considered to be a combination of an ordinary gene and one of these accessory elements (Brink, 1958). Change from variegated to red is explained by the removal of the element from the locus either by a process called transposition or by loss of the element from the genome. In transposition, the element moves from one locus to a second locus (McClintock, 1951). Transposition may lead to the origin of new mutable loci.

The Hfr_3 locus of *Escherichia coli* is similar to the *P* locus of maize. We may consider that the Hfr_3 locus has three alleles: *high rate of change*, F *present* (state I); *high rate of change*, F *absent* (state II); and *standard rate of change*. Like the variegated pericarp allele, the determinant of state I has a dual structure. One component is the gene for high rate of change. The other component is the *F* factor. Change from state I to state II may be explained by the removal of the *F* factor from the Hfr_3 locus. The consequence of this removal is the establishment of the infectious state in which the *F* factor multiplies autonomously.

The regular changes in state of $\mathring{\sigma}_3$ from *Hfr* to *F*+ and from *F*+ to *Hfr* parallel the variations in phase of certain *Salmonella* strains with regard to expression of flagellar antigens (Lederberg & Iino, 1956). In *Salmonella* this variation in phase depends on changes in state of a locus H_2 so that, when this locus turns 'on', the phase 2 antigen is expressed, while when this locus turns 'off', its epistatic action on another locus H_1 is thereby removed and the phase 1 antigen is expressed.

Episomic alternation offers a physical model for these changes in state at the H_2 locus.

The *V* locus of *Nicotiana* offers another example of reversible change of state at a single locus (Smith & Sand, 1957; Sand, 1957). v_S is an allele which promotes the synthesis of pigment in cells of the epidermis. v_s represents the inhibited condition of the same locus. Changes may occur from v_S to v_s and from v_s to v_S. These changes resemble the changes in state of the Hfr_3 locus from state II to state I and from state I to state II. The stable allele *V* may be compared to the allele for standard rate of change at the Hfr_3 locus.

Relation of the $\mathring{\sigma}_3$ system to the Lambda-K12 system

We have already considered that the Hfr_3 locus has three alleles. For certain purposes it is more instructive to consider that the Hfr_3 locus has two *stable*

alleles, high rate of change and standard rate of change, and to view the allele for high rate of change as a genetic system which exists in two states.

The Lambda-K12 system is another genetic system which exists in two alternative states. In the *lysogenic* state, the prophage is present on the chromosome near to *Gal*. In the *sensitive* state, it is not. Lysogenicity and sensitivity resemble states I and II of $\mathcal{J}_3$. The counterpart of standard rate of change is not known. These ideas are summarized in Table 9.

Table 9. *Relation of the $\mathcal{J}_3$ system to the Lambda-K12 system*

	$\mathcal{J}_3$	Lambda-K12
1. Gene relates to episome		
(*a*) episome integrated	state I	lysogenicity
(*b*) episome vegetative or absent	state II	sensitivity
2. Gene does not relate to episome	standard rate of change	—

One may thus think of lysogenicity-sensitivity as gene-determined in the same sense that change of state at the Hfr_3 locus is gene-determined. In this view, strain K12 would be a special strain of *Escherichia coli* which possesses a preferred chromosomal site for Lambda just as $\mathcal{J}_3$ is a special strain of K12 which possesses a preferred chromosomal site for *F*. Strains of *E. coli* lacking the preferred site for Lambda would appear not to be lysogenized by Lambda although lysogenization might be occurring with low frequency at a variety of sites.

SUMMARY

A variant strain of *Escherichia coli* K12 ($\mathcal{J}_3$) was obtained after ultra-violet irradiation. This strain differs from wild type in the possession of a preferred chromosomal site for the *F* factor.

When wild-type females are infected with the *F* factor they are converted to *F*+ males which carry the *F* factor in the infectious state. These *F*+ males (standard) change with low frequency to *Hfr* males which carry the *F* factor on the chromosome. When the variant female ($\mathcal{Q}_3$) is infected with the *F* factor it is converted to an *F*+ male (state II) which changes with high frequency to a specific *Hfr* male (state I) which carries the *F* factor at the preferred site (Hfr_3).

Male strains carrying the preferred site alternate between the specific *Hfr* state and the *F*+ state. The mechanism of this alternation is the back-and-forth shift of the *F* factor between its integrated state of multiplication at the Hfr_3 locus and its autonomous state of multiplication. This process is termed *episomic alternation*.

Episomic alternation offers a physical model for regular changes in state of the gene.

The Hfr_3 locus has two allelic forms, high rate of change and standard rate of change. The allele for high rate of change may be considered as a genetic system which occurs in two possible states, state I in which the *F* factor is present at Hfr_3, and state II in which it is not.

The author wishes to thank Dr W. Hayes for greatly improving the manuscript.

REFERENCES

ADELBERG, E. A. & BURNS, S. N. (1960). Genetic variation in the sex factor of *Escherichia coli*. *J. Bact.* **79**, 321–330.

BRINK, R. A. (1958). Mutable loci and the development of the organism. *J. cell. comp. Physiol.* **52**, Suppl. 1, 169–195.

CAVALLI, L. L., LEDERBERG, J. & LEDERBERG, E. M. (1953). An infective factor controlling sex compatibility in *Bacterium coli*. *J. gen. Microbiol.* **8**, 89–103.

CAVALLI-SFORZA, L. L. (1959). Recombination in bacteria. *Recent Progress in Microbiology* (ed. G. Tunevall), pp. 40–50.

HAYES, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 75–93.

HIROTA, Y. (1960). The effect of acridine dyes on mating type factors in *Escherichia coli*. *Proc. nat. Acad. Sci., Wash.*, **46**, 57–64.

JACOB, F. & WOLLMAN, E. L. (1956). Recombinaison génétique et mutants de fertilité chez *Escherichia coli*. *C. R. Acad. Sci., Paris*, **242**, 303–306.

JACOB, F. & WOLLMAN, E. L. (1958*a*). Genetic and physical determinations of chromosomal segments in *Escherichia coli*. *Symp. Soc. exp. Biol.* **12**, 75–92.

JACOB F. & WOLLMAN, E. L. (1958*b*). Les épisomes, éléments génétiques ajoutés. *C. R. Acad. Sci., Paris*, **247**, 154–156.

LEDERBERG, J. (1950). Isolation and characterization of biochemical mutants of bacteria. *Meth. med. Res.* **3**, 5–22.

LEDERBERG J., CAVALLI, L. L. & LEDERBERG, E. M. (1952). Sex compatibility in *Escherichia coli*. *Genetics*, **37**, 720–730.

LEDERBERG J. & IINO, T. (1956). Phase variation in *Salmonella*. *Genetics*, **41**, 743–757.

LWOFF, A. (1953). Lysogeny. *Bact. Rev.* **17**, 269–337.

MCCLINTOCK, B. (1951). Chromosome organization and genic expression. *Cold Spr. Harb. Symp. quant. Biol.* **16**, 13–47.

MCCLINTOCK, B. (1956). Intranuclear systems controlling gene action and mutation. *Brookhaven Symp. Biol.* **8**, 58–74.

RICHTER, A. (1957*a*). Complementary determinants of an Hfr phenotype in *E. coli* K12. *Genetics*, **42**, 391.

RICHTER, A. (1957*b*). Genetic recombination in *Escherichia coli* K12. M.S. Thesis, Univ. Wisconsin, Madison, Wisconsin, U.S.A.

RICHTER, A. (1958). Recombination analysis of mating type in *Escherichia coli* K12. *Proc. 10th int. Conf. Genet.* II, 232.

SAND, S. A. (1957). Phenotypic variability and the influence of temperature on somatic instability in cultures derived from hybrids between *Nicotiana langsdorfii* and *N. sanderae*. *Genetics*, **42**, 685–703.

SKAAR, P. D., RICHTER, A. & LEDERBERG, J. (1957). Correlated selection for motility and sex-incompatibility in *Escherichia coli* K12. *Proc. nat. Acad. Sci., Wash.*, **43**, 329–333.

SMITH, H. H. & SAND, S. A. (1957). Genetic studies on somatic instability in cultures derived from hybrids between *Nicotiana langsdor fii* and *N. sanderae*. *Genetics*, **42**, 560–582.

Genet. Res., Camb. (1961), **2**, *pp.* 346–360
With 3 *text figures*
Printed in Great Britain

The ecological genetics of growth in *Drosophila*

4. THE INFLUENCE OF LARVAL NUTRITION ON THE MANIFESTATION OF DOMINANCE

By FORBES W. ROBERTSON

Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, 9

(*Received* 4 *November* 1960)

1. INTRODUCTION

Earlier work has demonstrated an association between genetic behaviour and development in the variation of the body size of *Drosophila* (Robertson, 1959 *a*, *b*). Using the cellular make-up of the wing as index, it appears that statistically additive changes are associated with changes in cell number whereas non-additive genetic behaviour, such as that encountered with inbreeding, heterosis and the effects of exchanging chromosomes between lines, is generally accompanied by correlated changes in body and cell size when flies are grown on the live yeast medium. When a small strain, which had been selected for small cell size, was crossed to the foundation unselected population the F_1 was the same size as the latter and the genetic differences accumulated by selection behaved as recessive. Such behaviour presents a sharp contrast with the more or less additive behaviour of comparable differences, due to selection for body size, which, at least in the early stages of selection, leaves average cell size unchanged. But, at the same time, it recalls the comparatively recessive behaviour on the part of small lines which have been long selected for small body size (Robertson, 1954). To clarify the origin of such differences, further experiments have been carried out and they are dealt with in this paper.

Other tests have shown that the composition of the larval diet may greatly affect the expression of genetic differences which influence body size, especially when selection and/or inbreeding has been practised (Robertson, 1960; Prabhu & Robertson, in press) and so comparisons under different nutritional conditions are essential for a fuller understanding.

Two strains have been selected for small cell size in a population which is unrelated to those which were used in the earlier selection for cell size. Repeated crosses between the unselected population and the selected lines have been carried out both on the usual live yeast medium and also on various chemically defined, aseptic media which were made sub-optimal by reducing the level of some essential nutrient. To test for genetic interaction, chromosomes from the unselected population have been substituted in the genetic background of the selected lines and the results of these tests afford useful comparisons with earlier studies of the effects of chromosome exchange.

2. MATERIALS AND METHODS

The flies used in these experiments are all derived from a cage population of *Drosophila melanogaster*, known as Pacific; only females have been scored. Estimates of cell size refer to the average surface area of cells on one side of the wing; details of counting procedure and various tests of reliability have been given in an earlier publication (Robertson, 1959 *a*). Cell area is measured in squared hundredths of a millimetre, transformed to natural logarithms.

Body size is generally recorded as three times log thorax length, in $\frac{1}{100}$ mm.; differences on the log scale can be converted approximately into percentage differences by multiplying by 100. The method of measuring live flies and general cultural procedure have been described before (Robertson & Reeve, 1952). All tests have been conducted at 25° C. All variances have been multiplied by 10^4 to make comparisons easier.

Aseptic culture on chemically defined media has been used to control the larval diet, as described in an earlier publication (Robertson, 1960). Medium C of Sang (1956) has been taken as reasonably equivalent to a complete medium and sub-optimal diets have been prepared by reducing the level of either casein, which is the protein source, ribosenucleic acid or choline. Generally only one kind of nutrient was reduced at a time. For both live and aseptic media, four to five replicated cultures have been set up and eight to ten flies from each culture have been measured, so the mean sizes are each based on data from about forty flies.

Selection for small cell size has been carried out in two lines started at the same time from the Pacific population and treated throughout in the same way; they are referred to as lines A and B. Each generation, fifty pairs of flies, drawn equally from five replicated cultures, were scored for cell size, by removing and mounting one wing from each fly. The two pairs in each culture with the smallest cell size were combined to give ten pairs of parents per generation. Controls from the unselected population were set up at the same time and selection response is expressed as deviations from unselected. Thorax length was also recorded, either on all the females scored or on a representative sample to see how body size changed as selection proceeded.

For the many crosses, numerous progeny have been reared from the selected parents of a given generation to provide the parent flies of the cross. F_1 and parent strains were always set up at the same time under similar conditions. A single generation of relaxed selection apparently makes no difference to mean body size in the tests dealt with here. Various other experiments have been carried out but it is more convenient to deal later with the procedure in their case. We shall deal first with the selection response.

3. EXPERIMENTAL RESULTS

(*a*) *Selection for small cell size*

Figure 1 shows the response to selection as deviations from the unselected. Controls were not reared every generation. The upper graphs represent the changes

in cell size while the lower ones show what happened to body size. Not all generations are equally represented by deviations for both characters in the two lines. Thus, at generation 5, there was no record of cell size in Line A because the hatch was poor and selection was relaxed in this generation although the thorax length was measured. Also, at generation 7, although selection was carried out as usual in both lines, the controls were scored for body size only, due to pressure of work.

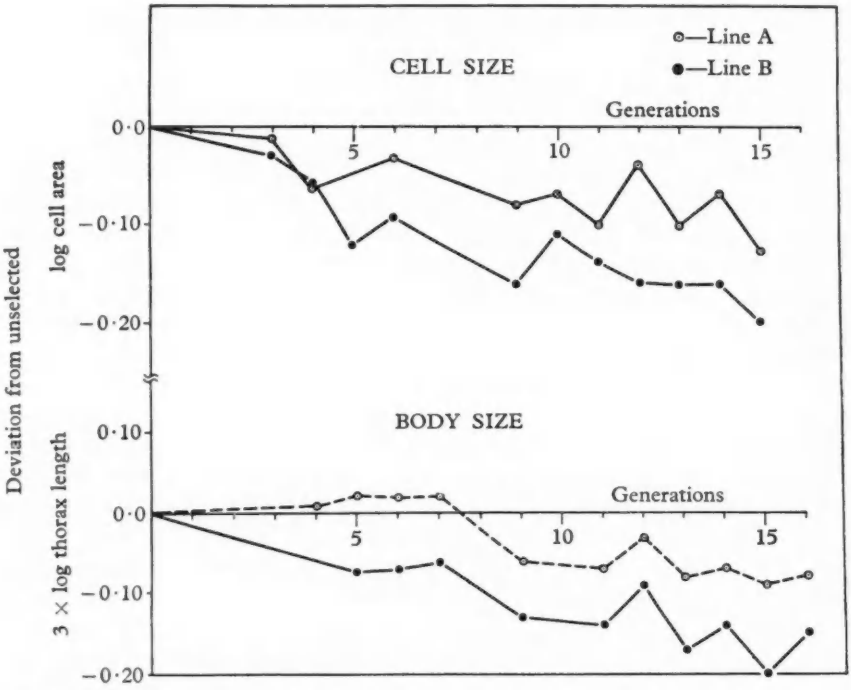


Fig. 1. The response to selection for small cell size. The upper curves show the changes in cell size; the lower ones show the changes in body size.

Selection led to an immediate decline in cell size which continued until about generation 10 and resulted in 10–12% reduction of cell area in Line A and 17–20% reduction in Line B. The decline in cell size was accompanied by a reduction in body size. This is shown in Fig. 2 in which the points for the two lines represent the average for the last few generations of selection. It should be noted in Fig. 1 that the initial response to selection for smaller cell size in Line A was unaccompanied by changes in body size which, however, declined later. In Line B there was an immediate decline in size. Lack of correlation between body and cell size can be attributed to variation in the inverse relations between cell size and number in the wing which can be responsible for considerable differences in

cellular make-up without corresponding changes in wing or thorax size (Robertson, 1959*a*). Evidently selection for this kind of change has been more effective in Line A than Line B.

In Fig. 2 body size is expressed in square measure—2 × log thorax length. If changes in wing cell size and number which are correlated with changes in body size, are accompanied by parallel changes in the rest of the exoskeleton, the graph

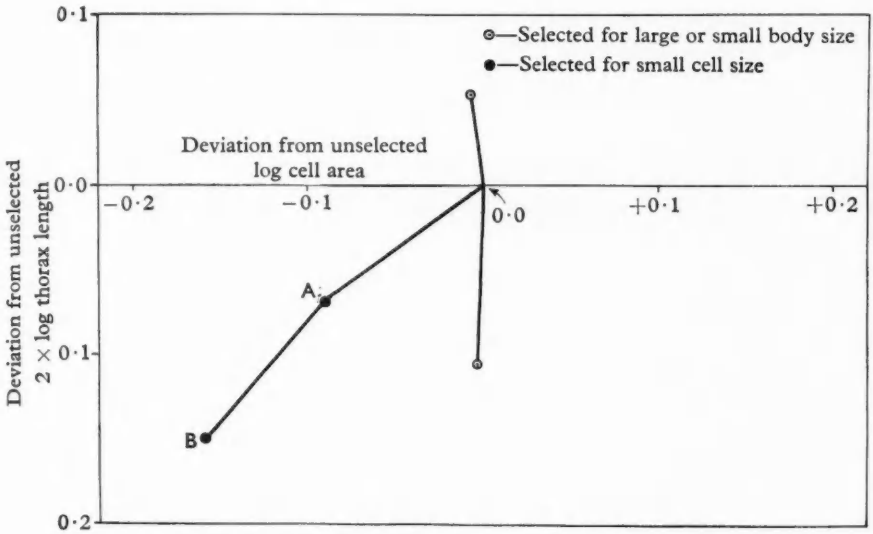


Fig. 2. The changes in body size which accompany selection for smaller wing cells are compared with the average effects on cell size of selecting for either large or small body size in several different populations.

suggests that the final reduction in body size is roughly proportional to the decline in wing cell size, and that changes in cell number are unimportant. Figure 2 also shows the average effects of selecting for large or small body size in several different populations; the data are taken from an earlier publication (Robertson, 1959*a*). Selection for change in body size does not alter average cell size, at least in the early stages of selection, whereas selection for small cell size certainly reduces body size.

(*b*) *Crosses on the live yeast medium*

A number of crosses between the selected lines and the unselected population have been carried out at generation 12 or subsequently, i.e. when there was little evidence of continuing response to selection. There is some additional evidence of the stability of average body size during this period since a subline was taken off both A and B lines at generation 15 and allowed to mate at random in bottles while selection was continued as usual. Controls were not scored for these additional generations of selection so they are not shown in Fig. 1. After four generations

of such relaxed selection there was no evidence of any change in the direction of more normal size (Table 1). The relaxed line from A was actually slightly smaller than the selected line. The controls were always drawn from the large cage population and it is assumed that their performance remained effectively constant over the period of these comparisons.

Table 1. *The effects of relaxing selection for four generations*

	Deviation from unselected—body size	
	Line A	Line B
Continued selection	−10·4	−18·7
Relaxed selection	−14·2	−18·2
Difference	−3·8*	0·5

* Indicates significance at the 0·05 level of probability.

Table 2 presents the results of the five crosses between Line A and the unselected population and the six crosses in which Line B was used. They are tabulated in order of increasing body size of the selected line, of which the smallest average size is reckoned as zero and the others are expressed as deviations from this figure. The reason for doing this will be considered later. In some of the Line B crosses the same parent flies provided eggs for separate lots of cultures which were set up several days apart. Hence the double entries for generations 13 and 17. Variation between the means for each parent type or cross is heterogeneous ($p < 0{\cdot}01$), so the nutritional conditions on the live yeast medium varied in successive tests.

Table 2. *The dominance deviation in successive crosses on the live yeast medium*

Body size of selected line—coded from smallest mean	Generation number	Differences		
		Unselected–selected	F_1–Mid-parent	Unselected–F_1
		Line A		
0·0	19	13·8	−1·8	8·7**
2·7	17	12·9	−0·3	6·1*
4·6	15	15·9	1·1	6·8**
9·7	18	12·8	2·1	4·3*
9·9	14	9·3	4·5*	0·1
		Line B		
0·0	15	19·2	5·6*	4·0*
3·1	13	16·8	3·4*	5·0*
3·4	17	15·4	7·2*	1·4
3·5	19	13·5	−0·1	6·6**
4·4	17	21·3	7·7*	3·0*
8·8	13	10·6	5·3*	0·0

* and ** indicate significance at the 0·05 and 0·01 levels of probability.

The third and fourth columns in Table 2 show respectively the deviations of the F_1 from the mid-parent value and the unselected parent. There is evidently great heterogeneity in the size of the F_1 relative to that of the parents. In the A crosses the average size of the F_1 coincides with that of the unselected parent in one test, while in the others it fluctuates about an intermediate level. In the B series, the F_1 is intermediate only in one cross. In all the others it either does not differ appreciably from the size of the unselected parent or significantly exceeds the mid-parent value.

Thus the F_1 is either the same size as or nearly as big as the unselected parent often enough to justify the earlier emphasis on the apparently recessive behaviour of the differences created by selection for small cell size. The new feature is the variable manifestation of the genetic behaviour. There is no evidence of correlation between the relative size of the F_1 and the chronological order of the crosses, and since temperature was kept constant there is little doubt that such differences are due to variation in the composition of the larval diet.

As noted above, there is statistically significant heterogeneity between mean size in successive tests for both lines and also the unselected population. How far the selected lines and the unselected population respond in the same way to this environmental variation can be inferred from the regression of the former on the latter. For Line A and the F_1 of the cross to unselected, the regressions work out at $0{\cdot}98 \pm 0{\cdot}40$ and $1{\cdot}34 \pm 0{\cdot}50$ respectively, while the corresponding regressions for Line B and the F_1 are $0{\cdot}06 \pm 0{\cdot}47$ and $1{\cdot}19 \pm 0{\cdot}37$. Thus only Line B shows possible departure from parallel fluctuation, although the difference in slope between it and the others does not exceed the 0·05 level of significance. Table 2 suggests that the positive departure from the mid-parent value in the crosses between Line A and unselected tends to increase under more favourable conditions which increase body size generally, so that the F_1 is intermediate, at one end, and the same size as the unselected, at the other end, of the 10% range in size. In the B series, the F_1 coincides with the unselected parent in the test in which the selected line attains largest size, but otherwise there is no evidence of a trend.

(*c*) *Crosses on sub-optimal diets*

To provide a further check on the influence of larval diet on the manifestation of apparent dominance, crosses have been carried out on aseptic synthetic media which comprise the so-called complete medium and several media in which the levels of either casein, RNA or choline have been reduced. The effects of these treatments are shown in Table 3. These tests were not all carried out at the same time. The relative order of the various treatments is indicated by how far they reduced the body size of the selected lines below the level attained on the complete medium.

It is quite clear that Line B suffers a relatively greater decline on all treatments than Line A. In the A crosses the F_1 does not differ significantly from the unselected population, either on the complete medium or on the medium with 3%

casein. But when the casein content is reduced to 2% or the RNA level is lowered to half (0·18%) or a quarter (0·09%) of the concentration in the complete medium, the F_1 does not differ significantly from the mid-parent value. In the B series, the F_1 is about the same size as the unselected on the complete medium and the media with 2% casein and 0·18% RNA, deviates significantly from the mid-parent value on the lowest concentration of RNA and actually exceeds the size of the unselected parents on the 3% casein diet. Finally on the low levels of choline the F_1 exceeds the size of the unselected parents in all four crosses, significantly so in three of them.

Table 3. *The dominance deviation in crosses on sub-optimal diets*

	A Crosses			B Crosses		
Medium	Deviation from complete medium	F_1–mid-parent	Unselected–F_1	Deviation from complete medium	F_1–mid-parent	Unselected–F_1
Complete	0·0	6·6**	−1·6	0·0	5·5**	−0·9
3% Casein	−13·1	4·3*	1·9	−10·0	−0·5	−4·1*
2% Casein	−22·8	0·7	7·3**	−28·5	10·3*	1·2
0·18% RNA	−5·0	−1·4	9·4**	−11·6	4·6*	1·6
0·09% RNA	−13·6	1·3	4·4*	−22·1	3·6*	5·1*
150μg. Choline	−18·6	11·2**	−6·1*	−28·2	15·0	−6·9**
100μg. Choline	−26·4	4·5	−2·0	−46·0	13·5	−7·5**

* and ** indicate significance at the 0·05 and 0·01 levels of probability.

These tests on different diets confirm that the chemical composition of the larval diet largely determines the relative size of the F_1 and hence the apparent genetic behaviour. But the distribution of differences is erratic and there is little evidence that conditions which lead to general decline in size, irrespective of the composition of the diet, are associated with a regular trend in apparent genetic behaviour. Thus in the A crosses, both the diets with 3% casein and 0·09% RNA lead to about 13% reduction in body size of line A but dominance was present in one case and absent in the other. Also, for both series, the effects of low levels of either casein or choline led to similar reduction in size but the relative position of the F_1 was quite different. Hence it is the specific, qualitative composition of the diet which matters rather than its quantitative effect on body size. The parallel behaviour, for both sets of crosses, on the low choline diet suggests that parallel selection for small cell size has lead to similar alterations in metabolism in the two lines.

(*d*) *Crosses to the Caribbean population*

As a further test of genetic behaviour, both lines have been crossed to an unrelated cage population known as Caribbean. The crosses were made after fourteen generations of selection and the flies were grown on the live yeast medium and on two synthetic diets which either lacked fructose or had a low (0·09%) RNA content, but otherwise had the same composition as the complete medium.

Table 4. *Comparisons of the body size of the Caribbean with the Pacific population and the selected lines*

	Deviation from Caribbean		
Genotype	Live yeast	No Fructose	Low RNA
A	−6·4**	−7·7**	3·7*
B	−11·4**	−8·1**	−3·0*
Pacific population	5·7**	4·5**	13·1**

* and ** indicate significance at the 0·05 and 0·01 levels of probability.

On the more favourable diets provided by the live yeast medium and the fructose-deficient diet, which leads only to minor reduction of body size, the Caribbean is some 5% smaller than the Pacific population. But with the lower RNA concentration the difference is increased to 13%, indicating a relatively greater requirement for RNA on the part of the Caribbean population. These differences are shown in Table 4 while the results of the crosses are given in Table 5.

Table 5. *Crosses of the selected lines to the unselected Caribbean population*

	A × C		B × C	
Treatment	F_1–Mid-parent	C–F_1	F_1–Mid-parent	C–F_1
Live yeast	6·1**	−2·9*	5·7*	0·0
No fructose	0·1	3·1*	5·5*	−1·4
Low RNA	10·1**	−11·9**	6·1*	−4·4**

A, B and C refer to the selected lines and the Caribbean population.
* and ** indicate significance at the 0·05 and 0·01 levels of probability.

When Line A was crossed to the Caribbean population, the F_1 was significantly larger than the unselected parents on the live yeast medium but intermediate when fructose was omitted from the diet. With low RNA concentration, the cross significantly exceeded the larger parent. In the B crosses, on both live yeast and fructose-deficient media, the F_1 was the same average size as the Caribbean population but, on the low RNA diet, exceeded it. The parallel behaviour on the low-RNA diet in which the F_1 of both crosses exceeded the larger parent may be attributed to the genetic background common to both the lines derived from the same foundation population. These comparisons provide further evidence that the relative size of the cross is influenced by the nature of the larval diet.

(e) The chromosome substitutions

If we bear in mind the complex interrelations of metabolic pathways in growth, the observed differences in genetic behaviour suggest that selection has led to changes at a number of loci and that interaction between non-allelic genes may be important. These inferences have been tested directly by substituting chromosomes from the Pacific population in the genetic background of each of the selected lines, and the effects of their single and joint effects have been compared. This procedure tests only for interaction between genes on different chromosomes but it has been found useful in earlier analyses of heterosis in crosses between inbred lines (Robertson & Reeve, 1954). Details of procedure and analysis have been given in the earlier paper and only the minimum needed for interpreting the present experiments are given here.

With the aid of marked, autosomal inversions which suppress recombination and a system of back-crossing, we can create genotypes in which single doses of chromosomes I, II or III from the unselected population are substituted in the background of the selected line. The small fourth chromosome is ignored. If the

Table 6. *The effects of substituting chromosomes from the unselected population in the background of the selected lines*

Genetic background	Least-squares estimates of chromosome effect			Interaction variance
	I	II	III	
A	0·3	4·1	1·9	3·8*
B	2·1	0·4	4·6	7·3**

* and ** indicate significance at the 0·05 and 0·01 levels of probability.

genetic constitution of the selected line is represented as AAA, the three alternative single substitutions may be represented as XAA, AXA and AAX, to show that one pair of chromosomes consists of homologues derived from different sources. Double substitutions are represented as XXA, XAX and AXX, while the triple substitution—the F_1 of the cross between selected line and unselected population—is XXX. Including the selected line there are altogether eight genotypes for comparison. The stages which preceded the final test were carried out on a sufficiently large scale to ensure the substitution of a representative sample of chromosomes from the unselected population.

The estimates of the average effects of replacing particular chromosomes and the test for non-additive interaction is based on a least-squares analysis. If the size of the selected line is represented as A, then the effects of replacing one member of each chromosome pair in turn by chromosomes from the unselected population may be expressed as A + a, A + b and A + c, while joint substitutions can be expressed as A + a + b, A + a + c, A + b + c and A + a + b + c. The resulting equations can be solved to give the least-squares estimates of the average effect and a test

for the presence of interaction, which will take the form of discrepancy between the effects of joint substitutions and the sum of their estimated individual effects when substituted one at a time. The error variance is the error variance of a mean, derived from the within- and between-culture components of variance.

The results are shown in Table 6. The distribution of genetic differences, due to selection, differ in the two lines. In A, selection has especially altered II, while in Line B it is chromosome III which differs most. Also in Line B there is an appreciable sex-linked difference which has been confirmed in the males by reciprocal crosses between Line B and the unselected population. Both sets of comparisons show evidence of significant interaction between chromosomes, and the level of interaction is greater for the substitutions in the B than in the A background. No doubt this is related to the more consistent tendency to recessive behaviour in crosses between Line B and the unselected population.

Table 7. *Comparison of the effects on body size of single and joint substitutions of chromosomes from the unselected population in the genetic background on lines A and B*

	Deviation from selected line, body size	
Chromosomes substituted	*A*	*B*
I	3·6	7·4
II	3·7	6·5
III	2·8	6·6
Sum of single effects	10·1	20·5
I+II+III	6·8	14·0
Difference	3·3	6·5

The general implication of these results can be more easily appreciated by a simpler approach which is summarized in Table 7. This shows the effects of making single substitutions in the background of either line and also the comparisons between the sum of these individual effects with their joint effect in the F_1 of the cross of each line to the unselected population. Any substitution from the unselected population tends to restore normal body size, but there is a kind of law of diminishing returns so that the sum of the single effects exceeds the joint effect by some 50% in both series. Further implications of these experiments will be considered later.

(*f*) *Selection from the F_2 of the cross between the lines*

To throw further light on the genetic behaviour of the differences due to selection, the two selected lines were crossed reciprocally at generation 15, and taken to a large F_2 from which two lines were started. Selection for large body size was carried out by selecting ten out of fifty pairs.

When the selected lines were crossed, the F_1 was significantly larger than the larger A parent—by about 5%—but was still some 10% below the level of the unselected population. In the F_2 there was a sharp decline to a level about halfway between the selected lines. Selection resulted in an immediate increase for three to four generations but no further progress thereafter and both lines fluctuated at a level which, on average, was a little below that of the unselected population.

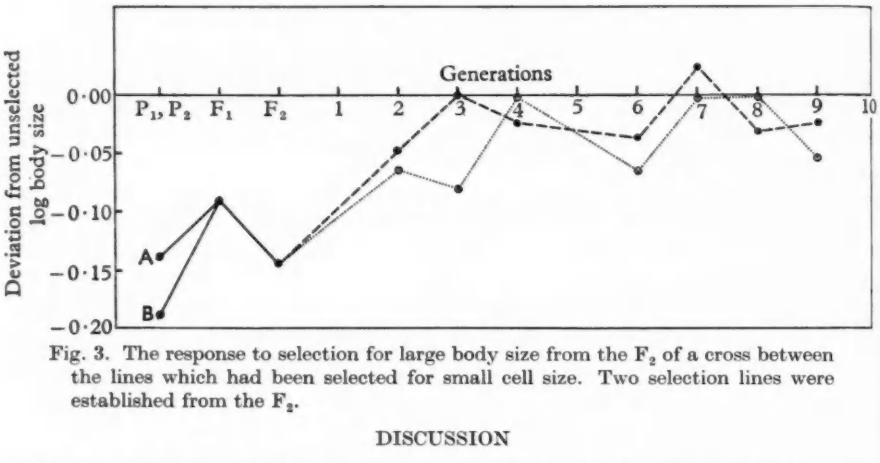


Fig. 3. The response to selection for large body size from the F_2 of a cross between the lines which had been selected for small cell size. Two selection lines were established from the F_2.

DISCUSSION

The most significant feature of these experiments is the tendency for differences in body size, which have been produced by selection for small cell size, to behave as recessive in crosses to the unselected population. Such behaviour appears to be quite general. It was reported in a similar selection experiment with an unrelated population (Robertson, 1959*a*). It also holds for crosses between the selected lines and the unrelated Caribbean population. The genetic behaviour of differences due to selection for small cell size differs from that shown by equally small or smaller lines produced by selection for small body size. When the latter are crossed to the foundation population the F_1 is intermediate or, where a positive deviation exists, it is relatively less than is generally true for lines selected for small cell size.

Selection for small cell size is not a particularly effective way to reduce body size. Thus, after some fifteen generations of selection, the two lines A and B were only about 10% and 15% smaller than the unselected population, whereas other experiments with the Pacific population, in which small thorax length has been selected for, have produced as great or greater deviations in five generations. Also, in other tests, in which small body size was selected for in several different populations, body size was reduced about 50% before the response ceased (Robertson, 1955). Part of such differences in response may be due to selection for change in the cell size and number relations in the wing, which can vary inversely within fairly wide limits without change of wing or body size (Robertson, 1959*a*). This was also illustrated in the early stages of selection in Line A.

These facts, together with the tendency for wing cell size to remain unchanged when selection for either larger or smaller body size is carried out, suggest that the physiological changes which reduce body and cell size together differ qualitatively from the changes produced by selection for smaller body size, at least in the early stages of selection, and that such differences are closely related to the differences in genetic behaviour.

But prolonged selection for smaller body size has produced evidence of genetic behaviour which approximates more closely to that which distinguishes lines selected for small cell size, namely comparatively recessive behaviour (Robertson & Reeve, 1954). Selection will, of course, involve a general loss of heterozygosity (Lerner, 1954), while evidence derived from the exchange of chromosomes between lines suggests that selection for smaller body size is especially likely to accelerate the trend to homozygosity (Robertson, 1954). It appears that selection for smaller cell size enables us to make the kind of changes in development which contribute especially to the later stages of selection for smaller body size. Equivalent reduction in size, due either to selection in a highly heterozygous background or to inbreeding decline, will be generally distinguished by absence or presence of correlation between the size of the body and that of the wing cells (Robertson, 1959 *b*). So, for a given reduction in size, selection for smaller cells is likely to involve a greater loss of heterozygosity than selection for smaller body size. This could help to account for the comparative ineffectiveness of selection for smaller cell size in reducing body size.

This view is consistent with the results of substituting chromosomes from the unselected population in the background of the selected lines. The type of interaction revealed by this analysis recalls the effects of exchanging chromosomes between inbred lines (Robertson & Reeve, 1954). These earlier experiments suggested that the stability of body size rests on dominance and complementary interaction between genes at different loci. It is interesting that Breese & Mather (1960) have recently reported a similar genetic situation with respect to viability and so such behaviour is likely to be general for any character which is subject to inbreeding decline. In the present tests, as in the earlier ones, the sum of the effects of the single substitutions considerably exceeded their joint effect. This pattern of diminishing returns is a practical demonstration that genes at different loci overlap in function to ensure normal growth, although it does not follow that their primary effects are the same.

The genetic behaviour of the differences produced by selection is greatly influenced by the composition of the larval diet. When the same parent strains are crossed the F_1 may be either midway between them or the same average size as the unselected parent in successive tests on the live yeast medium or on slightly different sub-optimal synthetic media. The comparisons on the various diets did not show any correlation between genetic behaviour and general quantitative effect on body size, except, possibly, for the tendency for the deviation from intermediacy to be greater in these tests on the live medium which lead to all round increase in size, in the crosses between Line A and the unselected population. The

specific composition of the diet determines whether the F_1 is to follow the metabolic pathways characteristic of the unselected parent or whether the genetic differences produced by selection are to influence development as well.

If we consider the interrelations of metabolic processes, the polygenic nature of the differences due to selection and also the influence of nutrition on genetic behaviour, it may be inferred that the apparently recessive behaviour is progressively established during the course of selection and that it depends, to no small degree, on the replacement of heterozygous by homozygous combinations. The population may carry individual recessive genes whose effects are so insensitive to variation either of genetic background or environment that they behave as recessive in virtually any situation. But there is no evidence that such genes were important in the experiments described here. If the reduction of body size by selecting for smaller cells is a rather effective way of disturbing the normal homeostasis of growth, genes or chromosomes segments which behave as recessive in the altered gene complex are likely to be fixed and such fixation will provide a more stable genetic background for further changes of this nature.

It is uncertain how far parallel selection in the two lines led to similar changes. On the one hand, the early differences in selection response between the lines, the different distribution of effects on the major chromosomes, the heterosis when the selected lines were crossed and the greater tendency for crosses between Line B and the unselected population to resemble the latter than was the case in the crosses between Line A and unselected together imply that numerous genetic differences exist.

On the other hand, on the choline-deficient media, when either Line A or B was crossed to the unselected population, the F_1 exceeded the latter. This could mean that selection led to similar changes in metabolism, although the genes responsible for such changes may occur at different loci. Such considerations are relevant to the response to selection from the F_2 of the cross between the lines. This was not very great and ceased rather abruptly after three to four generations. Parallel selection in the two parent lines could have fixed the same genes at a number of loci so that the genetic variance is low, although, as noted above, there is evidence to the contrary. It might also mean that physiological changes common to both parent lines and the lines derived from the F_2 set a limit to the increase in body size, even though genetic variation remains and could be utilized in the absence of this limitation.

SUMMARY

(1) Two lines have been selected for small wing cell size from the cage Pacific population. Body size was reduced by about 10% and 15% in the two lines which did not regress when selection was relaxed.

(2) The effects of crossing each line to the unselected population has been determined in a number of repeated tests on the live yeast medium and also on various sub-optimal synthetic media.

(3) The size of the F_1, relative to the size of the parents, is greatly influenced by the composition of the larval diet. The F_1 may coincide with the mid-parent value but generally significantly exceeds it and is often the same size as the unselected parent population.

(4) In crosses to an unselected population on alternative media the F_1 was either the same size as the unselected population or exceeded it.

(5) Crosses between the selected lines produced an F_1 which exceeded the larger parent but remained well below the level of the unselected population.

(6) To test for interaction between genes at different loci, chromosomes from the unselected population were substituted in the genetic background of each of the selected lines to provide an array of genotypes in which one, two or three pairs of major chromosomes had homologues derived from different strains. Least-squares analysis indicated differences between the lines in the distribution of effects among the chromosomes together with the presence of interaction between chromosomes and this was greater for the substitutions in the line which showed the greater consistency of recessive behaviour in crosses to the unselected population.

(7) At the end of the selection experiment two lines were selected for large body size from the F_2 of the cross between the two selected lines. Both responded to selection for three to four generations and then fluctuated at a level slightly below that of the unselected population.

(8) The physiological changes which involve correlated changes in body and cell size differ from those which result from selection for smaller body size, at least in the early stages of such selection, and are associated with differences in genetic behaviour. The apparently recessive property, which involves extensive non-allelic interaction, is progressively established during the course of selection. Apparently selection for smaller cell size is particularly effective in disturbing the normal homeostasis of growth and is accompanied by relatively greater loss of heterozygosis than is likely with equivalent reduction in size due to selection for smaller body as opposed to cell size.

I wish to thank Evelyn Davidson, Alexa Hamilton and Rosemary Singh for valuable technical assistance and also Mary Thompson for much processing of data. Thanks are also due to Mr E. D. Roberts for preparing the diagrams.

REFERENCES

Breese, E. L. & Mather, K. (1960). The organisation of polygenic activity within a chromosome in *Drosophila*. II. Viability. *Heredity*, **14**, 375–399.

Lerner, I. M. (1954). *Genetic Homeostasis*. Edinburgh: Oliver and Boyd.

Robertson, F. W. (1954). Studies in quantitative inheritance. V. Chromosome analyses of crosses between selected and unselected lines of different body size in *Drosophila melanogaster*. *J. Genet.* **52**, 494–515.

Robertson, F. W. (1955). Selection response and the properties of genetic variation. *Cold Spr. Harb. Symp. quant. Biol.* **20**, 166–177.

ROBERTSON, F. W. (1959 *a*). Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. *Genetics*, **44**, 869–896.

ROBERTSON, F. W. (1959 *b*). Studies in quantitative inheritance. XIII. Interrelations between genetic behaviour and development in the cellular constitution of the *Drosophila* wing. *Genetics*, **44**, 1113–1130.

ROBERTSON, F. W. (1960). The ecological genetics of growth in *Drosophila*. 1. Body size and developmental time on different diets. *Genet. Res.* **1**, 288–304.

ROBERTSON, F. W. & REEVE, E. C. R. (1952). Studies in quantitative inheritance. 1. The effects of selection of wing and thorax length in *Drosophila melanogaster*. *J. Genet.* **50**, 416–448.

ROBERTSON, F. W. & REEVE, E. C. R. (1954). Studies in quantitative inheritance. VIII. Further analyses of heterosis in crosses between inbred lines of *Drosophila melanogaster*. *Z. indukt. Abstamm.- u. VererbLehre*, **86**, 439–458.

SANG, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *J. exp. Biol.* **33**, 45–72.

Genet. Res., Camb. (1961), **2**, *pp.* 361–372
Printed in Great Britain

A tomato selection experiment

By NEIL GILBERT

John Innes Institute, Bayfordbury, Hertford, Herts.

(*Received* 28 *February* 1961)

This paper presents the results of an experiment involving the F_1, F_2, F_3 and F_4 of all crosses between eighteen English tomato varieties. Williams & Gilbert (1960) described the behaviour of the F_1's and F_2's in previous years. I am now concerned to compare the F_3's and F_4's, selected for early yield, with the parents, F_1's and F_2's. The purpose is to find out whether, at the beginning of a programme of crossing and selection, the F_1's and F_2's tell us any more than do the parents about the relative performance of the inbred lines finally obtained. If (as appears) they do not, the initial choice of parents for such a programme can be made solely on the basis of parental performance; it would be a waste of time to make test crosses merely in order to compare the 'combining abilities' of the possible parents.

1. DESIGN OF THIS AND OTHER SELECTION EXPERIMENTS

Even in the case of a population segregating for a single Mendelian gene, we are unable to predict the (stochastic) behaviour of any particular selected line. We can, however, consider the behaviour of a population of lines, each experiencing the same selection pressure. The average response to selection can then be compared with the theoretical prediction, using the variance between lines as the estimate of error. It seems to me, therefore, that a useful selection experiment must involve replication of distinct, similarly treated lines. Experiments to compare, say, one upwardly selected line with one selected downwards may have some practical interest but are of little use for theory. An ideal selection experiment would therefore start with an F_2 family (or several different F_2's), select from it a dozen plants to give F_3's, select (similarly) a dozen F_4's from each of those F_3's, and so on. We should also like to backcross each line to each parent in each generation. Clearly, the problem is what to leave out of the experiment. In the present case, I was concerned to compare the responses of crosses from different parents to a uniform selection pressure. The replication was therefore made between parents; each of the 153 crosses (excluding reciprocals) between the eighteen parents was represented by one line in each generation. In each cross, the highest yielding of twenty F_2 plants was chosen to give F_3 seed and, similarly, the best of twenty of these F_3 plants gave F_4 seed. The yields of individual F_3 and F_4 crosses are then of small significance in themselves, since the individual lines are not replicated; it is the average performance of each parent, as represented by that parent's seventeen crosses, that is meaningful. I chose the number 18 to give a reasonable number of degrees of freedom between parents. The actual parents employed, all standard English varieties, are listed by Williams & Gilbert (1960).

They were chosen by Mr L. A. Darby, tomato breeder at the Glasshouse Crops Research Station. They include both common varieties and some less often grown. The 153 F_1 crosses, the derived F_2, F_3, F_4 families and the eighteen parents were grown outdoors in 1960 in a 13×13 lattice square design in two replicates, there being two extra plots per block. Each plot consisted of twenty-four plants. Plots carrying the hybrids were split into four for generations. Plots carrying parents were split into two parts A and B, carrying plants of the same variety grown from seed taken in two different years. This was to test the occurrence of maternal effects caused by season, since the seed of the different hybrid generations were taken in different years. Thus each parent was represented by a total of forty-eight plants, and each generation of each cross by twelve plants. The total number of plants, 8208, could not reasonably be exceeded in an experiment wherein each individual plant had to be measured. In addition, a guard row was grown on the perimeter of the whole experiment. There were forty-six missing plants (mostly rogues but also some apparently normal plants which flowered well but set no fruit). They were evenly distributed between generations. The totals of sub-plots containing such plants were adjusted accordingly. For each plant in the experiment, the date of first flowering and the number and weight (oz.) of first three weeks' ripe fruit were recorded. Average fruit weights were determined from the sub-plot totals. All results given here are on a per-plant basis, and are corrected (where necessary) for rows and columns of the lattice square design.

One consequence of taking $k = 18$ parents and $n = 4$ generations is, that $(k-2)/4 = n$; this means that, with each parent replicated four times as often as each cross, the parental main effects (t) in each generation are estimated as accurately as one-half the means of the parental yields ($p/2$) (ignoring the correlation between the errors of sub-plots within one plot). This is useful, as a large part of the analysis is concerned with comparing t and $(p/2)$.

That the plants were grown at all is due to the painstaking work of the glasshouse and garden staff of this Institute. For the recording I was entirely dependent on the help of Mr W. Williams and members of the Department of Plant Breeding. Mr Williams made the selections from the F_3. By permission of Dr F. Yates, I was able to use the Rothamsted electronic computer for a large part of the analysis; this depended on the work of the engineering and punch staff of the Department of Statistics. Mere thanks are insufficient, for the experiment could not have been made without this help. I am grateful to Mr N. W. Simmonds, the Editor, and the Referee for their valuable suggestions.

2. REJECTION OF OBSERVATIONS ON FLOWERING DATE

Analysis of flowering date showed that it was significantly affected by various environmental factors. For this character there was an average difference between A and B parents (seed of the same varieties taken in different years); and this difference interacted with parents and with blocks. Blocks also interacted with generations. Consequently, the experimental results obtained depend on the particular blocks used in the experiment and on the particular year in which the

seed was taken. Evidently, flowering date in tomatoes is not a satisfactory character for this kind of experiment since it is at the mercy of such environmental disturbances. These results are therefore omitted from this paper. The other three measurements showed no such effects.

3. APPARENT SELECTION FOR HETEROZYGOSITY OF A MENDELIAN GENE

Two parents ('Exhibition' and 'Moneymaker') are homozygous for the recessive Mendelian gene *u* (uniform), which prevents 'greenback' discoloration of the fruit. This, incidentally, gives some check that the various crosses are true to label. The genotypes of the plants selected from F_2 and F_3 can be ascertained from their F_3 and F_4 progenies. In selecting for early yield, this allele was ignored. Other things being equal, we should expect a 1:2:1 ratio in the selected plants (as well as in each progeny before selection). In the total of 71 families segregating for *u*, there were 628 *U* and 218 *u* plants. χ^2 (1 d.f.) for a 3:1 ratio is 0·27. The plants selected from the F_2 families to give F_3 seed were in the proportion $4uu$: $22Uu$:$6UU$. From the twenty-two F_3 families still segregating for this gene, the selection was $1uu$:$17Uu$:$4UU$. There is some possibility of misclassification among the ten *UU* plants, for with a progeny of twelve the probability of classifying a *Uu* parent as *UU* is about 3·2%; but such misclassification would act *against* the observed excess of heterozygotes. The total segregation of $5uu$:$39Uu$:$10UU$ may possibly indicate some selection against *uu* genotypes, but even if we compare the *Uu*:*UU* frequencies for a 2:1 ratio there is still a decided excess of heterozygotes. Apparently, the *Uu* heterozygote has been unconsciously selected. This is rather surprising, as it seems to be a valid generalization (no more) that hybrid vigour in inbreeding organisms is of the dominance type and therefore fixable. Direct measurement of these three genotypes against similar genetic backgrounds would be necessary to establish firmly such a heterozygote advantage, if it exists. The effect might be due to linkage.

4. WITHIN-PLOT VARIANCES

Each generation of each cross was represented by six plants in each of two subplots, giving ten degrees of freedom for within-plot variance. The standard deviations were analysed as observations in their own right. It is more usual to analyse log variance, but this square-root transformation gives much the same answer and has the recommendation that it is the S.D. that is used in estimating percentiles. These figures are available for yield and fruit number only, since the fruit weights were estimated from sub-plot totals. Although the parents were grown in subplots of twelve plants each, the standard deviations quoted here are calculated within sets of six plants, and so are comparable with the S.D.'s of the other generations. A significant correlation was found between within-plot S.D. and plot mean. Part of the differences between the S.D.'s of different generations may therefore be explained away as a reflection of differences between generation means. The crude standard deviations have therefore been corrected for their

observed within-generations regression on the means (Table 1). Taking these figures at their face value, it appears that a conspicuous increase in variability in F_2 has almost disappeared by F_4, in which case little further advance under selection can be expected. Now parental 'main effects' can be estimated from the

Table 1. *Average within-plot standard deviations*

	Yield		Fruit Number	
	Crude	Corrected	Crude	Corrected
Parents	8·48	8·86	3·72	3·90
F_1	9·07	8·81	3·70	3·62
F_2	9·73	9·78	4·22	4·26
F_3	9·58	9·55	4·17	4·15
F_4	9·15	9·02 (± 0·18 approx.)	4·07	3·94 (± 0·08 approx.)

S.D.'s of the 153 crosses. It is found that there is strong parental control of the variability of the crosses. This may be partly explained by the similar effects on the means. The figures in Table 2 are therefore corrected as before for the regression on the mean. (This regression was found to be homogeneous between main effects and interactions; it showed no curvature.)

Table 2. *Analyses of variance of corrected within-plot S.D.'s*

		Mean squares			
	d.f.	F_1	F_2	F_3	F_4
Yield					
Main effects	17	9·24	8·09	8·73	4·92
Interactions	134	3·74	4·94	4·38	5·67
Fruit number					
Main effects	17	1·79	1·30	1·35	0·85
Interactions	134	0·82	1·09	0·99	1·26

These figures suggest that, even after allowing for the regression on the mean, there is some parental control of variability in yield but not in fruit number. This conclusion is supported by the correlations between these main effects and the S.D.'s of the parents themselves (Table 3).

Table 3. *Correlations* (16 *d.f.*) *between S.D. main effects and parental S.D.'s* (*both corrected for regression on mean*)

Parents ×	Yield	Fruit number
F_1	+0·262	−0·178
F_2	+0·345	+0·190
F_3	+0·264	−0·125
F_4	+0·422	−0·125

Now the parental and F_1 variabilities should be free from genetic segregation; and the average S.D.'s quoted behave accordingly. The parents transmit 'environmental' variability as well as average yield to their offspring. (The smallest parental yield S.D. was 5·8, and the largest, 10·3.) There is, of course, always the possibility that the parents were not completely homozygous.

5. GENERATION MEANS

Although, for each sub-plot, yield = fruit number × average fruit weight, these generation means naturally do not satisfy the same relation exactly. As mentioned in the previous paper (Williams & Gilbert, 1960), high early yield is associated with high fruit number rather than large fruit size. This has held true during selection, for the response in yield from F_2 to F_4 has been achieved by an increase in fruit

Table 4. *Generation means*

	Yield	Fruit number	Fruit weight
Parents	22·1	10·1	2·32
F_1	27·0	11·4	2·43
F_2	24·6	10·8	2·35
F_3	25·2	11·1	2·36
F_4	26·0	11·7	2·32
Mean top-parent	25·5	12·5	2·58
	(± 0·43*)	(± 0·19*)	(± 0·022*)
	(± 0·61†)	(± 0·25†)	(± 0·033†)

* S.E. of difference between two F means.
† S.E. of difference between parental and one F mean.
The 'top-parent' is the largest of the two parents in each cross.

number, the fruit weight remaining constant. (These responses to selection are of the order of magnitude predicted from the selection pressure—1 in 20 per generation—and the observed within-plot variances.) The F_1 hybrid vigour is relatively greater in yield than in fruit number or weight. This confirms the result of the previous paper, that—to a large extent—heterosis for yield results from the combination of one parent's high fruit number with another parent's high fruit weight.

6. ANALYSES OF VARIANCE

These analyses (Table 5) are made by fitting additive main effects (general combining abilities, g.c.a.) for each parent. The 17 d.f. for main effects are split into 1 d.f. for regression on parental means and the 16 remaining d.f. for deviations of the main effects from this regression. This 1 d.f. for regression of main effects on parental means is also the 1 d.f. for regression of each cross on its mid-parent.

Throughout this paper, the term 'interaction' is used in its statistical sense of a departure from additivity. As expected, the interactions (i.e. specific combining

abilities, s.c.a.) increase in size in F_3 and F_4, confirming that the individual F_3 and F_4 family means signify very little since they have responded unequally to selection. In other words, the data tell us nothing about the average response to selection of particular crosses (but they can tell us something about the average response of all crosses involving a particular parent). This is equally true of fruit weight, even though that character shows no *systematic* response to selection (section 9).

Table 5. *Analyses of variance*

		Mean square			
	d.f.	F_1	F_2	F_3	F_4
Yield					
Main effects (g.c.a.):					
Regression on parents	1	13393	21400	21409	19262
Remainder	16	703	382	643	624
Interactions (s.c.a.)	135	274	210	355	488
Error	608	169	169	169	169
Regression on parental differences	1	250	1	402	709
Fruit number					
Main effects:					
Regression on parents	1	7082·9	9979·5	11319·3	13384·8
Remainder	16	129·4	76·6	93·7	147·1
Interactions	135	59·1	40·8	81·4	120·9
Error	608	31·6	31·6	31·6	31·6
Regression on parental differences	1	304·6	0·1	206·4	234·0
Fruit weight					
Main effects:					
Regression on parents	1	14·4492	19·3526	17·7664	15·9304
Remainder	16	0·2181	0·2039	0·1683	0·2633
Interactions	135	0·0762	0·0982	0·1729	0·1956
Error	608	0·0751	0·0751	0·0751	0·0751
Regression on parental differences	1	3·5827	3·0830	1·6769	1·7316

The terms for 'regression on parental differences' are discussed in section 10.

7. INTERACTIONS

The correlations between interactions (special combining abilities) in different years and generations are small (Table 6). This confirms the finding (of the previous paper) that the interactions, although genetically significant, are unpredictable.

The 1960 correlations between F_1 and F_2 include an environmental component because the F_1's and F_2's occurred together on the same whole-plots. Since a correlation r only refers a fraction r^2 of the variance of one variate to the second variate, a correlation must in most circumstances be about $\frac{1}{2}$ or more before it can

Table 6. *Correlations between interactions* (*s.c.a.*) (134 *d.f.*)

Same generation in different years:

	F_1		F_2
	1957	1958	1958
		Yield	
1958	0·069		
1960	0·206	0·252	0·273
		Fruit number	
1958	0·017		
1960	0·150	0·162	0·256
		Fruit weight	
1958	0·179		
1960	0·150	0·009	0·004

Between F_1 and F_2 generations in the same year:

	1958	1960
Yield	0·152	0·484
Fruit number	0·062	0·498
Fruit weight	0·019	0·266

give worth-while prediction. Although these correlations are all positive, therefore, they are not large enough to give useful prediction. For this we must rely entirely on the main effects.

8. REGRESSION RELATIONS BETWEEN MAIN EFFECTS

The yield y of the cross between parents i and j is $y=m+t_i+t_j+t_{ij}$, where t_i, t_j are the parental main effects or general combining abilities. We have just seen that the interaction (specific combining ability) t_{ij} is unpredictable. I shall now consider how the main effects in different generations are related to each other and to the corresponding parental quantity $(p/2)$ ($\frac{1}{2}$ parental mean). The regression coefficient of t on $(p/2)$ is also the regression of individual cross yields on their mid-parental values. The regressions presented here are phenotypic regressions. It is possible to correct for error (assuming that genetical and environmental effects are additive) and so obtain 'genetic' regressions. The procedure is discussed in the appendix to Williams & Gilbert (1960). The resulting matrices are not positive definite (either because of sampling errors or because the assumption of additivity is wrong) and so the multiple regressions are nonsensical; while the simple genetic regressions (on one variate only) tell the same story as these phenotypic regressions. The S.S. for regression of each generation's main effects on parents have already been given in Table 5.

Table 7 is given *in extenso* to make the point that, for fruit weight (which is not responding consistently to selection), the preceding generation alone gives as good prediction as any, and better than the parents; whereas for fruit number and yield, predictions from the immediately preceding generation and

Table 7. *S.S. for regression of main effects (g.c.a.) on the main effects of previous generations*

Regression	S.S. absorbed by regression		
	Yield	Fruit number	Fruit weight
F_2 on F_1 and $\frac{1}{2}p$	23,000	10,500	20·8
F_1	18,300	9,800	20·0
$\frac{1}{2}p$	21,400	10,000	19·4
F_3 on F_2, F_1, $\frac{1}{2}p$	21,600	11,400	19·2
F_2, F_1	18,500	10,900	19·0
F_2, $\frac{1}{2}p$	21,600	11,400	19·0
F_1, $\frac{1}{2}p$	21,500	11,400	19·0
F_2	18,500	10,900	18·6
F_1	13,000	9,500	18·1
$\frac{1}{2}p$	21,400	11,300	17·8
F_4 on F_3, F_2, F_1, $\frac{1}{2}p$	21,300	14,000	18·7
F_3, F_2, F_1	18,800	13,800	18·7
F_3, F_2, $\frac{1}{2}p$	21,100	13,900	18·3
F_3, F_1, $\frac{1}{2}p$	21,200	14,000	18·7
F_2, F_1, $\frac{1}{2}p$	19,600	13,500	18·5
F_3, F_2	18,800	13,700	18·3
F_3, F_1	18,500	13,600	18·7
F_2, F_1	13,400	12,600	18·5
F_3, $\frac{1}{2}p$	20,700	13,900	18·1
F_2, $\frac{1}{2}p$	19,500	13,400	17·7
F_1, $\frac{1}{2}p$	19,600	13,400	18·4
F_3	18,400	13,600	18·1
F_2	13,400	12,500	17·6
F_1	8,100	10,200	18·2
$\frac{1}{2}p$	19,300	13,400	15·9

from the parental means are equally good (and supplement each other to some extent). Similar conclusions hold for the relations between the individual crosses in different generations (which is hardly surprising since we have seen that the interactions are poorly correlated). There is no sign of curvature in these regressions.

Table 8. *Coefficients of regression of main effects on parents*

Regression	Yield	Fruit number	Fruit weight
F_1 on $p/2$	0·66	0·68	0·73
F_2 on $p/2$	0·83	0·80	0·84
F_3 on $p/2$	0·83	0·86	0·81
F_4 on $p/2$	0·79	0·93	0·77

The values of these regression coefficients are not of great practical importance, in the sense that a breeder can only take the best that offers itself. The coefficients are all less than unity, confirming the result (of the previous paper) that although

the F_1 and F_2 generation means exceed the parental mean, the dispersion of the various crosses within the F_1 and F_2 generations is smaller than that of the parents themselves; and heterosis is rarely observed in crosses involving the best parents. The increase in size of these regressions from F_1 to F_2 is significant; I shall not attempt to interpret it in terms of dominance. The increase from F_2 to F_4 in the fruit-number regression coefficient, implying that the response to selection $F_4 - F_2$ is correlated with parental size, is not significant when tested against the appropriate error derived from $(F_4 - F_2)$ interactions (Table 10).

The 'remainder' terms in the analyses of variance in Table 5 show that the difference between the actual main effect and the value predicted by regression on the parent, is significant. But it is unpredictable (Table 9).

Table 9. *Correlations between deviations of main effects (g.c.a.) from parental regression* (15 *d.f.*)

Same generation in different years:

	F_1		F_2
	1957	1958	1958
		Yield	
1958	0·421		
1960	0·186	0·371	−0·091
		Fruit number	
1958	0·216		
1960	0·153	0·341	−0·035
		Fruit weight	
1958	0·397		
1960	0·253	0·439	0·225

Different generations in 1960:

		F_1	F_2	F_3
Yield	F_2	0·517		
	F_3	0·088	0·146	
	F_4	−0·182	−0·145	0·383
Fruit number	F_2	0·678		
	F_3	0·212	0·296	
	F_4	−0·039	0·150	0·471
Fruit weight	F_2	0·674		
	F_3	0·685	0·672	
	F_4	0·761	0·653	0·721

For fruit number and yield (which are responding to selection) there is good correlation between successive generations, but it is impossible to predict these 'deviations from regression' from F_1 to F_4. Fruit weight, which is not responding to selection, shows useful correlations between all generations. In spite of the deviant 1958×1960 F_2 figures, there is definite correlation between the same generation in different years.

9. RESPONSE TO SELECTION

From the practical point of view, the breeder must concentrate on the actual yield attained, rather than the increase achieved by selection. But it seems possible that the increase from F_2 to F_4 may lend itself to more sophisticated methods of prediction than can be used on the F_4 alone.

Table 10. *Analysis of variance of* ($F_4 - F_2$)

		Mean square		
	d.f.	Yield	Fruit number	Fruit weight
Mean difference between generations	1	3477	366·3	0·0505
Main effects:				
Regression on parents	1	28	124·7	0·0832
Remainder	16	574	95·9	0·0823
Interactions (=error)	135	315	69·2	0·1320

The interactions (specific combining abilities) M.S. is used as error because, as shown in section 6, individual crosses have responded unequally to selection. The 'regression on parents' M.S. provides a test of the significance of the difference between the F_4 and F_2 regression coefficients in section 8. In yield and fruit number there is some heterogeneity of main effects, i.e. the general combining abilities of different parents have responded differently to selection. Crosses involving one of the poorer parents might be expected to respond, on average, more than those involving one of the better parents; but there is no such effect. Nor are the responses correlated with the 'environmental' variabilities discussed in section 4 (or with the increase from F_1 to F_2 of such variability).

10. TOP-PARENT REGRESSIONS

The analysis by main effects (general combining abilities) is based on the assumption that the genetic contributions from the two parents are equally important to the determination of the yield of the cross. However, it is possible that the better parent will be more important in the event of upwards dominance or of upwards selection. Regression of the yields of crosses on the respective top- and bottom-parents is equivalent to regression on mid-parents and on the absolute difference between the two parents. As mentioned in section 6, the regression on mid-parent is part of the 'main effects' S.S., but regression on the parental difference is orthogonal neither to main effects nor to interactions. The M.S. for 'regression on parental difference' in section 6 represent the *additional* variance due to this source, i.e. they equal the S.S. for regression on top- and bottom-parents (2 d.f.) minus the S.S. for regression on mid-parents (1 d.f.). Now, in the appendix to the previous paper I found that, in the F_1 and F_2, the regression on parental difference was negligible in all cases except F_1 fruit number and yield. The present

results show that I was wrong to discount this exception; the F_1 yields and fruit numbers show general phenotypic dominance of the top-parent, the regression equation being

$$F_1 \text{ fruit number} = 0{\cdot}41 \text{ (top-parent)} + 0{\cdot}15 \text{ (bottom-parent)}.$$

This effect disappears in the F_2. A similar effect, ascribable to the upwards selection, appears in the F_3 and F_4: e.g.

$$F_4 \text{ yield} = 0{\cdot}48 \text{ (top-parent)} + 0{\cdot}22 \text{ (bottom-parent)},$$
$$F_4 \text{ fruit number} = 0{\cdot}53 \text{ (top-parent)} + 0{\cdot}30 \text{ (bottom-parent)}.$$

Fruit weight, which alters little from generation to generation, shows phenotypic dominance of small fruit size. There was a similar, but non-significant, tendency in 1957 and 1958. An obvious objection is that, since such asymmetry occurs, the analysis by main effects (g.c.a.) is no longer the best way of looking at the situation. This is true, but the analyses of variance of yield and fruit number in section 6 show that it is only just true, for the 'regression on parents' M.S. is far larger than the 'regression on parental differences' M.S., even though the latter may be significantly large on occasion. For fruit weight, on the other hand, the analysis could be improved by weighting the parental main effects. I have not done so here because this character showed no consistent response to selection anyway. It is interesting to note that, although the F_1 crosses have on average a greater fruit weight than their parents, their distribution about that average tends to follow the smaller parent. At the moment we cannot predict *how much* more important the top-parent will be; until we can, these general combining abilities (main effects) are the best that we can do.

11. CONCLUSIONS AND SUMMARY

All 153 crosses between 18 tomato varieties were grown in F_1, F_2, F_3 and F_4. The F_2, F_3 and F_4 were derived by selfing one plant of the previous generation. The F_2 plant chosen to give the F_3 was selected (1 in 20) for early yield; and the F_3 plant chosen to give the F_4 was similarly selected. Flowering date was an unsatisfactory character. In crosses segregating for the Mendelian gene 'uniform', a significant excess of heterozygotes was selected. The parents transmitted variability of yield (as well as average yield) to their offspring. The division of yield into its components fruit number and fruit size is useful because (1) much of the heterosis in yield can be viewed as a combination effect between these components, (2) the components responded differently to selection, (3) different components showed phenotypic dominance in different directions. The average yield and fruit number responded as expected to selection; fruit size did not. The F_1 generation means for all three characters exceeded the parental means; but the crosses were grouped more compactly about their generation mean than were the parents, so that heterosis rarely occurred in crosses involving the best parents. The yields of each cross were analysed into parental main effects (general combining abilities)

and interactions (specific combining abilities). No useful prediction of interactions could be made in any generation, either from the same generation in different years or from different generations in the same year. The main effects (general c.a.) were analysed into a part due to regression on parental yield, and a deviation from that regression. No useful prediction of the deviations from parental regression could be made in generations which had responded to selection. The actual advance under selection of different crosses, although not uniform, was unpredictable. During advance under selection, the parental means gave predictions of the (relative) performance of each generation's crosses which were as good as predictions based on the previous generation. This may, of course, be connected with the fact that the parents were inbred and that the amount of heterozygosity decreases in each successive generation. These results indicate, therefore, that in an inbreeding species propagated by seed, the early hybrid generations tell us nothing more than do the parental yields about the relative performance of the inbred lines that can be selected from those hybrids. (This generalization from *Lycopersicon esculentum* to inbreeders as a whole may, of course, be false.) The relative performance of F_1 hybrids, on the other hand, is better predicted from other F_1 crosses involving the parents concerned than from the yields of those parents. There was phenotypic dominance of high yield, large fruit number and low fruit weight. The extent of this dominance was not enough to invalidate the analysis by general combining abilities; and since it varied from year to year and from generation to generation, the emphasis that should be given to the top-parent (in contrast to the bottom-parent) in predicting the yields of crosses after selection, is as yet unpredictable.

REFERENCE

WILLIAMS, W. & GILBERT, N. (1960). Heterosis and the inheritance of yield in the tomato. *Heredity*, **14**, 133–149.

Genet. Res., Camb. (1961), **2**, *pp.* 373–383
With 2 plates
Printed in Great Britain

Timing imbalance in the meiosis of the F_1 hybrid *Oryza sativa* × *O. australiensis*

By S. V. S. SHASTRY and D. R. RANGA RAO
Division of Botany, Indian Agricultural Research Institute, New Delhi, India

(*Received* 28 *February* 1961)

The F_1 hybrid *Oryza sativa* ($2n = 24$) × *O. australiensis* ($2n = 24$) was secured and studied by Gopalakrishnan (1959). The study of meiosis in this hybrid assumed special significance in view of the controversy regarding the taxonomic position of *O. australiensis*. The observation of Shah (1955) that *O. australiensis* and *O. perennis* ($2n = 24$) are similar in having perennial rhizomatous stems and large spikelets and anthers was responsible for the inclusion of both these species in the section Sativa (Ghose, Ghatge & Subrahmanyan, 1956). Gopalakrishnan (1959) re-examined the morphology of *O. australiensis* and considered that this species is intermediate between the sections Sativa and Officinalis since whorling of panicle branches characteristic of *O. officinalis* is present in the former species. Further, while a maximum of four bivalents were recorded by him in the F_1 hybrid *O. sativa* × *O. officinalis*, a maximum of eight bivalents were recorded in the F_1 hybrid *O. sativa* × *O. australiensis*. Richharia (1960), in his treatise on inter-specific relations in the genus *Oryza*, supported Gopalakrishnan's view and suggested that *O. australiensis* might have originated by hybridization between the members of the sections Sativa and Officinalis in Australia.

Korah (1961) and Sampath (personal communication) observed that the chromosomes of *O. australiensis* were larger than those of *O. sativa* at somatic metaphase. Gopalakrishnan (1959) and Shastry & Rao (1961) reported that the bivalents of *O. australiensis* appeared to be larger than those of *O. sativa*. The comparison of total chromatin lengths at pachytene (Shastry, Rao & Misra, 1960; Shastry & Rao, 1961), however, revealed that the species *O. sativa* and *O. australiensis* have comparable lengths (*ca.* 400 μ) at this stage. Further, the pachytene bivalents of *O. australiensis* were highly heterochromatic. Shastry & Rao (1961) concluded that apparent largeness of the bivalents of *O. australiensis* is due to their undergoing less condensation (because of greater amount of heterochromatin) from pachytene to metaphase I stages.

The observation of a maximum number of eight bivalents in the F_1 hybrid *O. sativa* × *O. australiensis* (Gopalakrishnan, 1959) and the inference of hybrid origin of *O. australiensis* (Richharia, 1960) were considered unlikely by the present authors for the following reasons:

1. *O. australiensis* is endemic to Australia. No other species of *Oryza* belonging to either of the sections Sativa or Officinalis is reported from Australia.

2. There is no evidence of human introduction of rice into Australia except in very recent times.
3. The karyotype of *O. australiensis* is highly symmetric and the pachytene bivalents of this species are highly heterochromatic, a situation not yet discovered in any other species of the genus (Shastry & Rao, 1961).

A reinvestigation of the meiosis of the F_1 hybrid *O. sativa* × *O. australiensis* was therefore considered necessary. Since the parents differed in the sizes of their chromosomes at metaphase I, it was hoped that it would be possible to distinguish between auto- and allosyndesis, an aspect not studied by Gopalakrishnan (1959). To avoid the discrepancies in pairing due to varietal differences in parents, the F_1 hybrid obtained and studied by Gopalakrishnan (1959) was secured by the courtesy of Dr R. H. Richharia, Director, Central Rice Research Institute, Cuttack, and was used in the present investigation.

Spikes of suitable stage were fixed in acetic-alcohol (1:3 by volume) to which traces of ferric chloride were added. After fixation for 24 hours at low temperature (10–14° C.), the material was transferred to 70% ethyl alcohol in which it was stored. Simple aceto-carmine smears were employed for the study of meiosis. Temporary slides were used for photomicrography.

OBSERVATIONS

The meiotic data of this hybrid were based upon the examination of 223 PMCs at meta-anaphase I, 44 at diakinesis and 10 at diplotene. The chromosomes of *O. australiensis* are so distinctly larger and darker stained than those of *O. sativa* that, at all stages of meiosis, they could be distinguished (Plate I, Figs. 2, 5; Plate II, Figs. 8, 12, 13). The meiotic stages from pro-metaphase to late anaphase I were indistinguishable because of the high frequency of univalents, and consequently what is described as meta-anaphase I represents a heterogeneous group of PMCs.

1. *Pairing at prophase*

Several PMCs at pachytene stage were analysed in the F_1 hybrid, in which the univalent nature of the chromosomes was clear. In the ten PMCs analysed at diplotene stage, only twelve configurations were visible, all of which were univalents. It was inferred that they corresponded to the complement of *O. australiensis*, for reasons to be discussed later. In all the forty-four PMCs of early and late diakinesis that were analysed, the chromosomes of *O. australiensis* were distinct by their darker staining and larger size (Plate II, Fig. 8). No true allosyndetic bivalents were noted in any of these PMCs. In fifteen PMCs at diakinesis, two autosyndetic bivalents of *sativa* were recorded. The most significant feature of this stage was the variable number of visible univalents. While in every one of these forty-four PMCs the full complement of *australiensis* chromosomes was recognizable as univalents, the number of *sativa* chromosomes that were visible (those which have been sufficiently condensed) was variable. A variable number of non-chiasmatic associations have been noted at this stage (Plate II, Fig. 8).

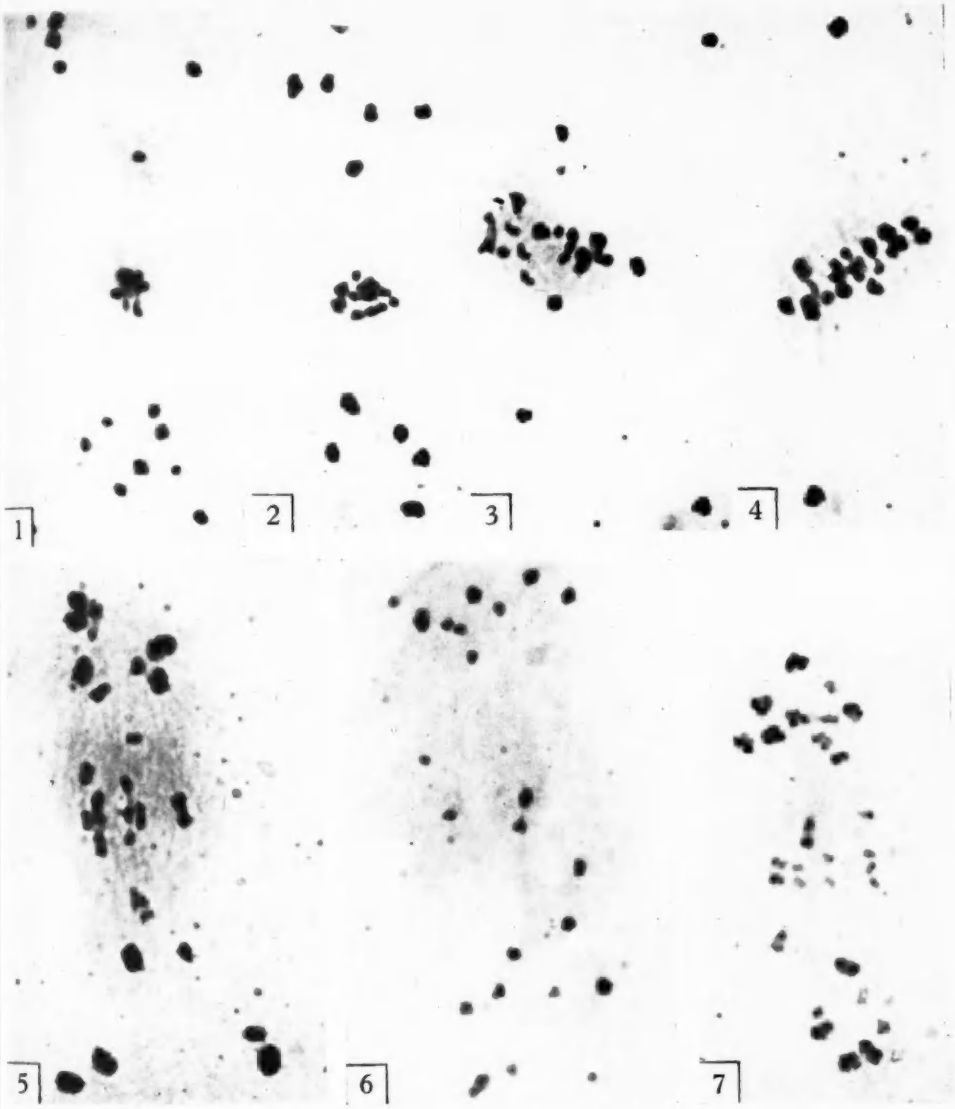


Fig. 1. Meta-anaphase I. Two autosyndetic bivalents, one e-e allo-association and two *sativa* univalents at equator. × 1100.

Fig. 2. Meta-anaphase I. Full complement of *O. sativa* and two univalents of *O. australiensis* at equator. × 1100.

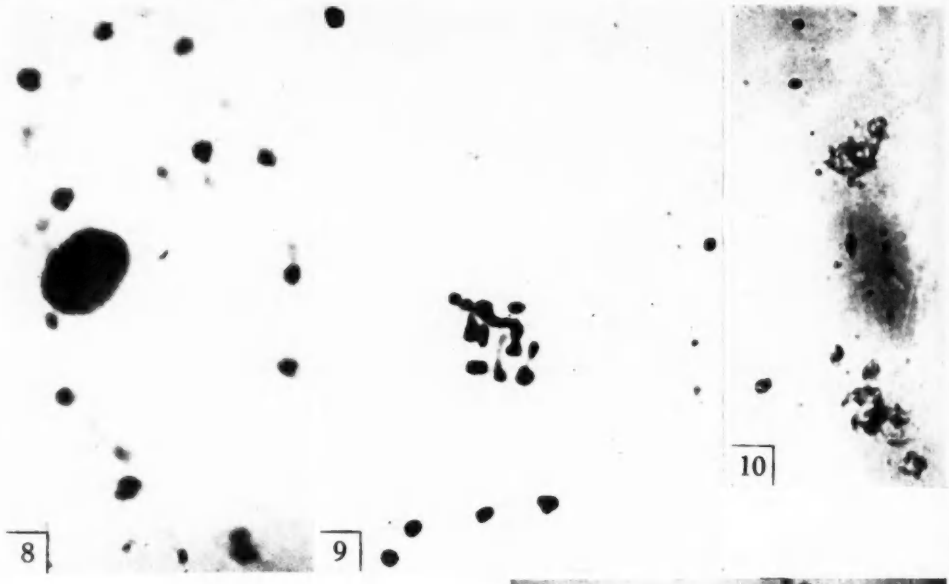
Fig. 3. Meta-anaphase I. Note the congression on the equatorial plate with four e-e allo-associations. Four *australiensis* univalents migrated to poles. × 1100.

Fig. 4. Meta-anaphase I. Four e-e allo-associations. Two *australiensis* univalents at the poles. × 1100.

Fig. 5. Meta-anaphase I. Ten *australiensis* and four *sativa* chromosomes at poles. *Sativa* univalents at equator dividing. × 1500.

Fig. 6. Meta-anaphase I. One lagging e-e allo-association. × 1400.

Fig. 7. Meta-anaphase I. Full complement of *O. australiensis* at poles. *Sativa* univalents at equator dividing. × 1100.



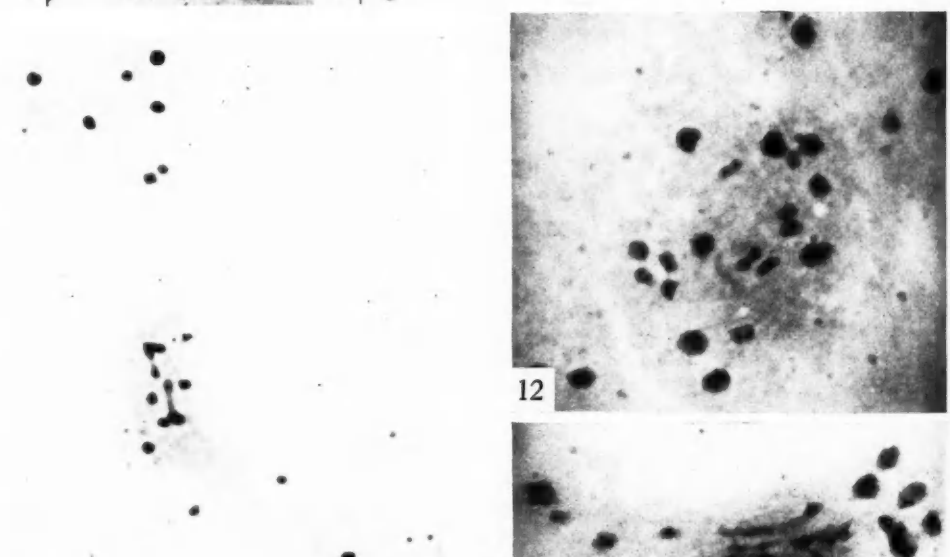


Fig. 8. Diakinesis. Note the chromosomes of *O. australiensis* which are stained darker and larger. Five univalent pairs secondarily associated. × 1200.

Fig. 9. Meta-anaphase I. Five e-e allo-associations at equator. Five *australiensis* univalents at poles. × 1400.

Fig. 10. Anaphase I. Note the wide differences in the condensation between the univalents. × 1400.

Fig. 11. Meta-anaphase I. Two e-e allo-associations at equator. Ten *australiensis* univalents at poles. × 1800.

Fig. 12. Metaphase I. Polar view. Twelve large *australiensis* univalents. Four divided and eight undivided *sativa* univalents. × 1500.

Fig. 13. Meta-anaphase I. Full complement of *O. australiensis* at poles. Three pairs of univalents of *O. sativa* formed false bridges. × 1500.

The number of univalents visible in different PMCs at this stage and the number which were secondarily associated are presented in Table 4. It could be clearly seen that the chromosomes of *sativa* were not all condensed at the same time while those of *australiensis* were more uniform in this respect. Further, the residual homology between the genomes seemed to permit a high degree of secondary association.

2. *Meta-anaphase I*

Out of 223 PMCs studied at this stage, 194 had twenty-four univalents each (Plate I, Fig. 3). In the remaining 29 PMCs, 1, 2 and 3 autosyndetic bivalents involving the chromosomes of *O. sativa* were recorded in 24, 4 and 1 PMCs respectively (Table 1, and Plate I, Fig. 1). In a single PMC, what appeared to be an

Table 1. *Frequency distribution of PMCs with autosyndetic bivalents and univalents at meta-anaphase I of the* F_1 *hybrid* O. sativa × O. australiensis

	Number of autosyndetic bivalents				
	O. sativa			*O. australiensis*	
Twenty-four univalents	1	2	3	1	Total
194	23	4	1	1	223

autosyndetic bivalent involving the chromosomes of *O. australiensis* was recorded. All the autosyndetic bivalents were rod-shaped with a single chiasma each. No true bivalent involving the chromosomes of the two species (allosyndetic) was recorded in any one of the PMCs scored. While in a majority of the PMCs no regular equatorial plate was formed, in twenty (8·6%) PMCs all the univalents were congressed on an equatorial plate (Plate I, Figs. 3, 4). In fifteen (6·4%) PMCs, while the entire complement of *O. australiensis* were at the poles, univalents and autosyndetic bivalents of *O. sativa* were aligned on the equatorial plate (Plate I, Figs. 5, 7; Plate II, Fig. 13).

The most frequent associations met with during meiosis in the hybrid appeared to be non-chiasmatic and they were arranged on the equatorial plate. The frequency of these pseudobivalents varied from 0 to 7, the modal value being 2 per PMC. The frequency distribution of the PMCs with varying numbers of pseudobivalents is given in Table 2. Autosyndetic pseudobivalents were not observed in any one of the PMCs. The pseudobivalents were all end-to-end associations (Plate I, Figs. 3, 4, 6; Plate II, Figs. 9, 11).

Table 2. *Frequency distribution of PMCs with various number of allosyndetic end-to-end pseudobivalents in the* F_1 *hybrid* O. sativa × O. australiensis

Number of allosyndetic, end-to-end pseudobivalents								
0	1	2	3	4	5	6	7	Total
122	21	40	23	12	3	1	1	223

3. *Distribution of univalents*

Of the 223 PMCs studied at meta-anaphase I, 142 could be clearly classified for the contribution of univalents at the poles. In all these PMCs, with the exception of five, univalents were arranged in three groups, one each at the two poles and a group of laggards at the equator (Plate I, Figs. 1, 2). The total number of *sativa* and *australiensis* chromosomes at the two poles is presented in a two-way table (Table 3). The frequency in each cell refers to the number of PMCs with the

Table 3. *Frequency distribution of* sativa *and* australiensis *chromosomes at both the poles of the meta-anaphase I PMCs in the* F_1 *hybrid* O. sativa × O. australiensis

Number of *sativa* chromosomes at both the poles	Number of *australiensis* chromosomes at both the poles													
	0	1	2	3	4	5	6	7	8	9	10	11	12	Total
0		1	2	—	5	4	7	7	7	7	10	2	5	57
1					1	5	5	2	3	2	3	2	1	24
2						3	1	2	3	2	1	1	1	14
3				1	—	2	1	—	2	4	3	1	1	15
4				1	—	—	—	1	3	4	2	2	1	14
5							1	—	3	1	1	3	1	10
6								2	2	—	—	—	—	4
7												1	—	1
8								1						1
9											1			1
10												1		1
Total		1	2	2	6	14	15	15	23	20	21	13	10	142

corresponding number of *sativa* and *australiensis* chromosomes. It will be seen from this table that while there were several PMCs (fifty-seven) with 1–12 chromosomes of *O. australiensis* at the poles, but no chromosomes of *sativa*, the reverse situation where one or more *sativa* chromosomes were present to the exclusion of *australiensis* chromosomes was not met with. In view of the large sample that has been employed, this could not be due to chance. Evidently, the chromosomes of *australiensis* either migrate to the poles earlier than those of *sativa* or fail to congress more frequently on the equatorial plate. The full congression on the equatorial plate in 8·6% PMCs can be accounted for by either of the causes.

In nine PMCs, 1–4 *australiensis* chromosomes migrated to poles with 0 or 1 chromosomes of *sativa*. These PMCs might represent early stages of meta-anaphase. In eighteen PMCs, 6–12 chromosomes of *australiensis* were observed along with 6–12 chromosomes of *sativa* and these PMCs might correspond to the late stage of meta-anaphase I. With the exception of these twenty-seven (19·0%) PMCs (Table 3), the two-way table clearly reveals the asymmetry in distribution of the chromosomes indicating either early migration to the poles or failure of congression at the equator of *australiensis* chromosomes (Plate I, Figs. 2, 5, 7; Plate II, Fig. 13). Further, the *sativa* univalents were dividing in some PMCs

(Plate I, Fig. 7) and exhibited stickiness in others (Plate II, Fig. 13). Wide differences in condensation of the univalents were observed in some cells (Plate II, Fig. 10).

Table 4. *Frequency distribution of univalents of* O. sativa *visible at diakinesis and attached to those of* O. australiensis *in the* F_1 *hybrid* O. sativa × O. australiensis

Number of *sativa* chromosomes secondarily associated with those of *australiensis*	Number of *sativa* chromosomes visible in addition to the full complement of *australiensis* chromosomes											
	2	3	4	5	6	7	8	9	10	11	12	Total
0											6	6
1											1	1
2	1											1
3					1							1
4						1					3	4
5					1		2		4		2	9
6						1	2		2		5	10
7						1	2				3	6
8							1				4	5
9												—
10											1	1
Total	1	—	—	—	2	3	7	—	6	—	25	44

DISCUSSION

1. *Nature and extent of pairing*

Gopalakrishnan (1959) recorded a maximum of eight bivalents (3·49% PMCs) and a maximum of twenty-four univalents (6·99% PMCs) with a mean pairing of $0{\cdot}06_{IV} + 0{\cdot}1_{III} + 3{\cdot}83_{II} + 15{\cdot}80_{I}$ per PMC in the hybrid *O. sativa* × *O. australiensis*. This suggests considerable lack of homology between the genomes, though more than between the genomes of *O. sativa* and *O. officinalis*. The cytological analysis done in the present investigation, on the other hand, gave no evidence of pairing leading to chiasma formation between the genomes of *O. sativa* and *O. australiensis*, the mean autosyndetic bivalent frequency being 0·15 per PMC and that of non-chiasmatic pseudobivalents, 1·06 per PMC. This discrepancy cannot be accounted for by the varietal differences since the same hybrid as was studied by Gopalakrishnan (1959) was employed in the present study. Although environmental factors do modify chiasma (thereby bivalent) frequency, such a wide discrepancy in observations cannot be attributed to this cause alone. It is, therefore, most likely that many of the pseudobivalents were scored as bivalents by Gopalakrishnan. In support of such a view, two points seem significant:

(i) While the present authors recorded 1–7 pseudobivalents per PMC in 43·3% of the cells, Gopalakrishnan made no mention of their occurrence.

(ii) Many of the drawings of PMCs with 'bivalents' are quite unconvincing as regards the chiasmatic connexion between the chromosomes and are comparable to allosyndetic pseudobivalents scored by the present authors.

2. *Autosyndetic pairing and its significance*

The occurrence of two bivalents in haploid variants of *O. sativa* (Morinaga & Fukushima, 1934; Hu, 1958) and *O. glaberrima* has been hitherto explained on the basis of the hypothetical secondarily balanced allotetraploid origin (Nandi, 1936) of the diploid species of *Oryza*. If the basic chromosome number for the tribe as postulated was 5, the haploids of the species with $2n = 24$ are expected to form two bivalents. Since it is established that no pairing occurs between the genomes of *O. sativa* and *O. australiensis*, the interspecific hybrid between these species offers an opportunity to test the expectations of autosyndetic pairing on the basis of alteration of the basic number from 5 to 7.

With the exception of the occurrence of a single PMC each (0·043 %) with three autosyndetic *sativa* and one autosyndetic *australiensis* bivalents, most commonly one (9·87 % PMCs) or two (1·7 % PMCs) autosyndetic bivalents involving *sativa* chromosomes were found in the F_1 hybrid *O. sativa* × *O. australiensis*. Consequently, the occurrence of autosyndetic bivalents in *O. sativa* and their non-occurrence in *O. australiensis* raised severe doubts regarding their being valid indicators of basic chromosome number in *Oryza*. This observation might mean either that (1) chromosome structural changes subsequent to alteration of the basic number from 5 to 7 have destroyed the homology between the chromosomes originally identical in *O. australiensis*, or (2) the bivalents in haploid *O. sativa* and the autosyndetic bivalents in the hybrid under study are the result of structural changes and do not point to the secondarily balanced allotetraploid nature of these species. If the former alternative is considered to be true, it is most surprising that the homology between duplicated chromosomes could be preserved in a species with a high variability and exposure to natural and human selection (*O. sativa*) and is lost in a species which is more primitive, with very limited geographic distribution (endemic to Australia), and is highly uniform (*O. australiensis*). On the other hand, it is more likely that the autosyndetic bivalents are the result of structural differences in *O. sativa* which is highly evolved. This interpretation, however, does not contradict the allopolyploid origin of the diploid species of *Oryza*, but it only emphasizes their functional diploid nature. Non-complementation for viability of the gametes in the desynaptic interspecific hybrid (*O. sativa* × *O. officinalis*) with a high degree of numerical regularities at anaphase I (Shastry, Sharma & Rao, 1960) likewise points to the same conclusion.

3. *Timing imbalance in the condensation of chromosomes*

Several cases exist in the literature (Darlington, 1937) where one or more chromosomes of a species are either retarded or advanced in condensation with reference to others at metaphase I. These differences in timing of condensation of chromosomes, referred to as timing imbalance, are often used as indications of allopolyploid origin of the species. Implicit in such an argument is that either the genomes of the related species differ in the duration of meiosis or that the different genomes interact in a hybrid so as to produce such an imbalance. Meiosis

of the F_1 hybrid *O. sativa* × *O. australiensis* offers an excellent example of such a situation.

The occurrence of twelve chromosomes of *O. australiensis* alone at the onset of diplotene, followed by a varying number of *sativa* univalents at diakinesis and probable early migration of *australiensis* univalents to the poles, clearly indicates the timing imbalance for condensation and mobility of the chromosomes of these two genomes. This is so striking that in 97·85% of the PMCs at meta-anaphase I, laggards remain at the equator, the composition of laggards being predominantly *sativa* chromosomes and a few *australiensis* chromosomes. These observations indicate one of the following possibilities:

1. The meiotic cycle of *O. australiensis* is much shorter than that of *O. sativa*.
2. The presence of *australiensis* chromosomes delays the condensation of *sativa* chromosomes; or
3. The presence of *sativa* chromosomes hastens the condensation of *australiensis* chromosomes.

It is not possible to distinguish between these alternatives, nor is it possible to decide whether these differences are intrinsic to genomes or the result of their interaction.

4. *Significance of pseudobivalents*

The use of the terms 'pseudobivalents' (Håkansson, 1940; Walters, 1954), 'quasi-bivalents' (Östergren & Vigfusson, 1953), 'associations not due to chiasmata' (Shastry, Sharma & Rao, 1961) and 'secondary pairing' (Darlington, 1928; Lawrence, 1929), and their classification into 'end-to-end', 'side-by-side' and 'end-to-side' types (Person, 1955), was made necessary because of the limited sense in which the term 'bivalent' was applied, and because of the concept that bivalent formation is indicative of homologous pairing at prophase followed by persistent chiasma formation at the stage of analysis (diakinesis or metaphase I). Several hypotheses have been advanced to explain the occurrence of pseudobivalents—(i) breakage and reunion of chromosomes originally brought into contact by chance, with no dependence on homology (Walters, 1950); (ii) matrical connexions between univalents (Walters, 1954); (iii) suspected pairing at early prophase followed by failure of chiasma formation (s-s associations; Person, 1955), and (iv) heterochromatic fusion, stickiness and non-specific attractions (Riley & Chapman, 1957; Natarajan & Swaminathan, 1958). Secondary pairing or secondary association, in its strict sense, pertains to inter-bivalent attraction which is apparent in the post-synaptic phase, not accompanied by actual visible connexions, and is related to residual homology in higher polyploids (Darlington, 1937), although similar attractions between univalents can be visualized. Person (1955) attached special significance to the sites of attachment between univalents of pseudobivalents. He observed a negative correlation between the number of s-s associations and true bivalents, indicating a competition between them. No such correlation was observed of e-s and e-e associations with true bivalents,

indicating that the latter two forms of pseudobivalents do not depend upon homology.

Although the pseudobivalents reported in the present study of the F_1 hybrid *O. sativa* × *O. australiensis* resemble the e-e associations of Person (1955) and pseudobivalents of Walters (1954), they are yet distinctive in the following respects from all the previously reported cases: (i) allosyndetic pairing is exclusively in the form of pseudobivalents and consequently it is not possible either to study the correlation between the true bivalents and pseudobivalents (Person, 1955) or estimate the 'potential bivalents' (Gaul, 1959); (ii) all pseudobivalents are allosyndetic, which indicates that whatever might be the cause of their origin, they are not random associations; (iii) all pseudobivalents conform to the end-to-end arrangement of Person (1955); (iv) the parental species are distinctive in their karyotypes, one (*O. australiensis*) being exceedingly heterochromatic; and (v) their occurrence is coupled with a distinct timing imbalance in condensation and possibly in migration between the chromosome complements of the parental species.

It is hazardous to consider that any one of the views expressed (Walters, 1954; Person, 1955; Riley & Chapman, 1957) on the origin of pseudobivalents are conclusive and of universal applicability. The interpretation of the pseudobivalents of the F_1 hybrid *O. sativa* × *O. australiensis* is further complicated by the five special features accompanying their detection enumerated above. The strongest points in favour of their reflecting homology between the genomes are that all of these associations are allosyndetic and that timing imbalance does not provide the necessary conditions for the formation of true bivalents. The weakest points of their being indicators of homology are that all of them are end-to-end associations and that the karyotype of one of the species (*O. australiensis*) has extensive heterochromatic segments, which can exhibit stickiness. The present evidence does not make it possible to choose between these two alternatives.

5. *Genetic differentiation and hybrid sterility in* Oryza

Absence of true allosyndetic bivalents in the F_1 hybrid *O. sativa* × *O. australiensis*, in the light of the suggested timing imbalance, can be interpreted to mean either that no homology exists between the chromosome complements of the constituent species or that the necessary conditions for pairing and persistence of chiasmata are not provided by the superimposition of the timing imbalance during meiosis. Preference for either of these alternatives is not justified by the present evidence.

Complete sterility is reported in the F_1 hybrids *O. sativa* × *O. officinalis* and *O. sativa* × *O. australiensis* (Gopalakrishnan, 1959). In the former hybrid, pairing at pachytene was complete followed by desynapsis in later stages of meiosis (Shastry, Sharma & Rao, 1960). In the latter hybrid, reported here, the timing imbalance followed by infrequency of complete separation of the full complement of the parental species to the poles at anaphase I is the possible reason for sterility. High sterility in inter-subspecific hybrids of *O. sativa* have already been demon-

strated by pachytene analysis to be due to chromosomal differentiation (Yao, Henderson & Jodon, 1958; Shastry & Misra, 1961). Other interspecific hybrids of the genus have thus far not been subjected to critical analysis. It is interesting to note that different mechanisms control hybrid sterility in the genus *Oryza*.

6. *Taxonomic position of* O. australiensis

The controversy regarding the taxonomic status of *O. australiensis* is largely due to its resemblance to *O. perennis* in having a perennial branched rhizomatous stem, bold grains and long anthers, and to *O. officinalis* with regard to the whorling of the branches in the panicle and the size of the chromosomes. This intermediate morphological appearance could be attributed to two causes:

1. *O. australiensis* could have been the progenitor of members belonging to both the sections Sativa and Officinalis, which have been subject to divergent evolution with regard to the distinguishing characters. If this hypothesis is true, the species *O. australiensis* might represent one branch in the evolution and isolation of the pre-Sativa and pre-Officinalis members of *Oryza*.
2. *O. australiensis* is of more recent origin, as a result of hybridization between the well-differentiated members of the sections Sativa and Officinalis. Richharia (1960) and Gopalakrishnan (1959) favoured the latter view, while the present authors are inclined to support the former view for the following reasons:

   (i) The karyotype of *O. australiensis* is highly symmetric, being classed in 2b of Stebbins' (1958) classification in contrast to *O. sativa* classifiable in 3c (Shastry & Rao, 1961). Such a high degree of symmetry is least expected in a highly evolved species.

   (ii) Shastry & Rao (1961) observed that pachytene bivalents of *O. australiensis* are unmistakably distinct from those of *O. sativa* and *O. officinalis* in their 'differentiated' appearance (Hyde, 1953). Primitive taxa of several other genera (cf. Stebbins, 1950, and Venkateswarlu, 1961) are characterized by excessive heterochromatic segments in their karyotype.

   (iii) The occurrence of a higher frequency of bivalents in *O. sativa* × *O. australiensis* than in *O. sativa* × *O. officinalis* (Gopalakrishnan, 1959) is not substantiated in the present study.

SUMMARY

The meiosis in the F_1 hybrid *Oryza sativa* × *O. australiensis* was studied. Contrary to the observations of Gopalakrishnan (1959), true allosyndetic bivalents were not found at metaphase I. The most frequent associations were non-chiasmatic, end-to-end pseudobivalents. Autosyndetic bivalents were recorded mostly in the complement belonging to *O. sativa*, which are distinguishable by their smallness and lighter staining. The meiotic cycle exhibits timing imbalance with

earlier condensation, and possibly migration, of the univalents belonging to *O. australiensis*. The data on meiotic pairing in the F_1 hybrid and the comparative morphology of *O. sativa*, *O. officinalis* and *O. australiensis* inicate that the last species is the most primitive member, having originated from the pre-Sativa and pre-Officinalis complex.

We are grateful to Dr B. P. Pal, Director, and Dr A. B. Joshi, Dean, Indian Agricultural Research Institute, for providing the facilities. We are also grateful to Dr R. H. Richharia, Director, Central Rice Research Institute, for providing the material, and to Dr M. S. Swaminathan for valuable discussion.

REFERENCES

DARLINGTON, C. D. (1928). Studies in *Prunus*, I and II. *J. Genet.* **19**, 213–256.

DARLINGTON, C. D. (1937). *Recent Advances in Cytology*, 2nd ed. London: Churchill.

GAUL, H. (1959). A critical survey of genome analysis. In *Proc. First International Wheat Genet. Symp., Winnipeg, Canada*, pp. 194–206.

GHOSE, R. L. M., GHATGE, M. B. & SUBRAHMANYAN, V. (1956). *Rice in India*. Indian Council of Agricultural Research, New Delhi.

GOPALAKRISHNAN, R. (1959). Cytogenetical studies on interspecific hybrids in the genus *Oryza*. (Unpublished thesis.) Indian Agricultural Research Institute, New Delhi.

HÅKANSSON, A. (1940). Die Meiosis bei verschiedenen Mutanten von *Godetia whitneyi*. *Lunds Univ. Arsskrift*, N.F. Adv. 2, **36**, 1–37.

HU, C. H. (1958). Studies on chromosome complement of cultivated rice. *J. agric. Ass. China* (Chinese), **21**, 11–23.

HYDE, B. B. (1953). Differentiated chromosomes in *Plantago Ovata*. *Amer. J. Bot.* **44**, 809–815.

KORAH, M. (1961). Cytotaxonomy and evolutionary trends in some species and varieties of *Oryza*. *Proc. 48th Indian Sci. Congr. Roorkee, India*, Abstracts, p. 306.

LAWRENCE, W. J. C. (1929). Genetics and cytology of Dahlia species. *J. Genet.* **21**, 125–159.

MORINAGA, T. & FUKUSHIMA, E. (1934). Cytogenetical studies on *Oryza sativa* L. I: Studies on the haploid plant of *Oryza sativa*. *Jap. J. Bot.* **7**, 73–106.

NANDI, H. K. (1936). Chromosome morphology, secondary association and origin of cultivated rice. *J. Genet.* **33**, 315–336.

NATARAJAN, A. T. & SWAMINATHAN, M. S. (1958). Haploidy induced by radiations in wheat. *Experientia*, **14**, 336–337.

ÖSTERGREN, G. & VIGFUSSON, E. (1953). On position correlations of univalents and quasi-bivalents formed by sticky univalents. *Hereditas*, **39**, 33–50.

PERSON, C. (1955). An analytical study of chromosome behaviour in a wheat haploid. *Canad. J. Bot.* **33**, 11–30.

RICHHARIA, R. H. (1960). Origins of cultivated rices. *Indian J. Genet.* **20**, 1–14.

RILEY, R. & CHAPMAN, V. (1957). Haploids and polyhaploids in *Aegilops* and *Triticum*. *Heredity*, **11**, 195–207.

SHAH, S. S. (1955). Morphological and anatomical studies in the genus *Oryza*. (Unpublished thesis.) Indian Agricultural Research Institute, New Delhi.

SHASTRY, S. V. S., RAO, D. R. R. & MISRA, R. N. (1960). Pachytene analysis in *Oryza*. I: Chromosome morphology in *Oryza sativa*. *Indian J. Genet.* **20**, 15–21.

SHASTRY, S. V. S., SHARMA, S. D. & RAO, D. R. R. (1960). Cytology of an inter-sectional hybrid in *Oryza*. *Naturwissenschaften*, **24**, 608–609.

SHASTRY, S. V. S. & MOHAN RAO, P. K. (1961). Pachytene analysis in the genus *Oryza*. IV: Karyomorphology in *O. australiensis*, *O. glaberrima* and *O. stapfii*. *Proc. Indian Acad. Sci.*, Section B (in press).

SHASTRY, S. V. S. & MISRA, R. N. (1961). Pachytene analysis in *japonica-indica* rice hybrids. *Curr. Sci.* **30**, 70–71.

Stebbins, G. L., Jr. (1950). *Variation and Evolution in Plants.* New York: Columbia University Press.

Stebbins, G. L., Jr. (1958). Longevity, habit and release of variability in the higher plants. *Cold Spr. Harb. Symp. quant. Biol.* **23**, 365–378.

Venkateswarlu, J. (1961). Cytotaxonomic studies in Coix. In *Symp. Cytogenetical Evolution of Angiosperms, Proc. 48th Indian Sci. Congr. Roorkee, India.*

Walters, M. S. (1950). Spontaneous breakage and reunion of meiotic chromosomes in the hybrid, *Bromus trinii* × *B. maritimus*. *Genetics*, **35**, 11–37.

Walters, M. S. (1954). A study of pseudobivalents in meiosis of two interspecific hybrids of *Bromus*. *Amer. J. Bot.* **41**, 160–171.

Yao, Y., Henderson, M. T. & Jodon, N. E. (1958). Cryptic structural hybridity as a probable cause of sterility in intervarietal hybrids of cultivated rice, *Oryza sativa* L. *Cytologia*, **23**, 46–55.

Genet. Res., Camb. (1961), **2**, *pp.* 384–393
With 3 *text-figures and* 1 *Plate*
Printed in Great Britain

Genetical studies on the skeleton of the mouse

XXIX. PUDGY

BY HANS GRÜNEBERG
Experimental Genetics Research Unit (Medical Research Council)
University College, London

(*Received* 6 *March* 1961)

INTRODUCTION

In previous papers of this series, ten genes of the mouse with major effects on the axial skeleton have been described. In two of them (curly-tail, *ct*/*ct*; Grüneberg, 1954*a*; Tail-short *Ts*/+; Deol, 1961) the involvement of the skeleton was clearly secondary. In congenital hydrocephalus (*ch*/*ch*; Grüneberg, 1953), the anomalies of the axial skeleton were part of a systemic effect on the membranous skeleton as a whole. In Danforth's short-tail (*Sd*/+; *Sd*/*Sd*; Grüneberg, 1958*a*), in Brachyury and Anury (*T*/+; *T*/*T*; *T*/*t*; Grüneberg, 1958*b*) and in Pintail (*Pt*/+; *Pt*/*Pt*; Berry, 1960), the axial anomalies were traceable to the notochord as the source of trouble (though in the first two of these, the notochord itself may be involved as part of a more fundamental disturbance of the primitive streak). In vestigial-tail (*vt*/*vt*; Grüneberg, 1957), anomalies of the distal end of the neural tube appeared simultaneously with a gross reduction of the ventral ectodermal ridge of the tail, the causal relationship between the two events remaining in doubt. In tail-kinks (*tk*/*tk*; Grüneberg, 1955*a*) and in undulated (*un*/*un*; Grüneberg, 1954*b*), the axial anomalies are the result of faulty differentiation of the sclerotomes. The only gene so far studied in this laboratory which seems to implicate segmentation as such is Bent-tail (*Bn* ♂; *Bn*/+ ♀; Grüneberg, 1955*b*) in which the distal tail vertebrae are increasingly reduced in size and, in more extreme cases, in number. However, in this case the interpretation is based on the findings in the adult skeleton and hence carries less conviction than the embryological 'eye-witness accounts' in the other mutants. The condition to be described in this paper profoundly upsets segmentation and leads to a chaotic situation of the whole vertebral column.

GENETICS

The recessive gene 'pudgy' (symbol *pu*) has possibly been induced by X-rays. It appeared in the later descendants of a ♂ derived from the 'specific locus' experiments carried out in the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A., under the direction of W. L. Russell. According to results by W. St Amand and M. B. Cupp (to be published), pudgy is located in Linkage Group I, the order of markers being *pu*–*p*–c^{ch}. The present investigation was carried out on the descendants of a group of animals handed over to me for study by L. B. Russell in July 1958.

Pudgy mice can easily be identified at birth. Their tail is reduced to a small stub or all but absent, and the whole trunk region is considerably shortened. As the limbs are of about normal length, the adult pudgy mouse has a somewhat toad-like appearance. As pudgies are very poor breeders, the stock is being maintained by $+/pu \times +/pu$ matings. Twenty such matings with at least twenty classified young each produced 773 normal and 208 *pu/pu* offspring. There is thus a significant deficiency of recessives which is obviously due to reduced viability (81% at birth); there is further selective mortality in the nest and after weaning.

Embryonic material was collected by mating normal sisters of pudgies to known $+/pu$ ♂♂. The litters ranged in age from 9 to 19 days, and during this whole of this period, *pu/pu* embryos can easily be identified by their external appearance. Sixty-five litters of embryos thus obtained included 450 normal and 70 *pu/pu* individuals. On the 5:1 expectation, there is thus again a significant deficiency of recessives (70 as compared with 450/5 or 90), the prenatal viability being 70/90 or 78%, in good agreement with that found at birth. However, this good agreement may be spurious. It results from the fact that there are 33 segregating : 32 non-segregating litters instead of something much closer to a 2:1 ratio, a discrepancy presumably due to an accident of sampling. Considering only those litters which contained at least one *pu/pu* embryo, these included 216 normal and 70 *pu/pu* embryos. Allowing for the selection inherent in these data, the expected number of recessives, on a 3:1 expectation, is 78·585. The observed deficiency of recessives is thus no longer significant ($\chi^2_1 = 1{\cdot}293$), and the possibility must be considered that the viability of *pu/pu* may be normal or nearly so before birth, selective elimination of pudgies happening at birth or soon afterwards.

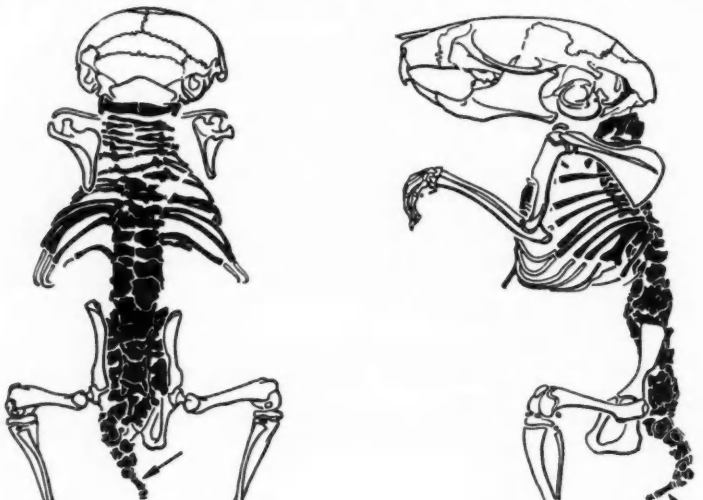
ADULT ANATOMY

The anatomy of pudgy has been studied in alizarin clearance preparations (21 *pu/pu* and 13 normal litter mates). In addition, papain macerations of the skulls of six *pu/pu* and of six normal litter mates have been studied.

Anomalies of an extreme kind are found from one end of the vertebral column to the other, in the ribs and in the sternum; anomalies are also often detectable in the skull in the immediate vicinity of the foramen magnum. Otherwise the skull and the appendicular skeleton, including the girdles, appear to be quite normal. A typical specimen is shown in Text-fig. 1. The general type of anomaly of the vertebral column is very uniform in all pudgies examined. But as the state of the axial skeleton can only be described as chaotic, no two pudgies agree in detail. The vertebral column is considerably shortened all along its length; it consists of a jumble of vertebrae, and fragments of vertebrae, which are irregularly fused with each other in such a complex way that it is impossible to discover in any given mouse how many elements have gone into the making of such a telescoped structure with all its irregular kyphoses and lordoses. As is to be expected, the ribs are as irregular as the vertebrae. Particularly in the caudal half of the thoracic region, the ribs tend to be bunched together, sometimes as many as six successive ones, with irregular fusions which are sometimes superficial, but often so complete

that it is difficult to decide whether a given rib is simple or composite in nature. In the sternum, fusions between adjacent sternebrae, often at an angle, are common.

More detailed study shows that vertebrae, or parts of vertebrae, often retain some of their regional specificity, such as the characteristic shape of the transverse processes in the lumbar and sacral region. Very exceptionally, a whole vertebra has escaped from the surrounding chaos, such as the caudal marked by an arrow in Text-fig. 1. Occasionally, the number of elements in a particular



Text-fig. 1. Pudgy ♀, 23 days old. Drawings based on photographs. A single virtually normal caudal vertebra is indicated by arrows.

region of the spine can be ascertained. Combining such counts from a number of pudgies, it seems probable that the jumble of ossicles and fused bony masses is, in fact, derived from the normal number of segments, or something fairly close to it, as far down as the os sacrum. In the caudal region, the number of segments represented is certainly much less than the normal average of thirty and presumably very variable.

Inevitably, adjacent soft structures like muscles, nerves and blood vessels will be involved in this tangle, but for technical reasons these have not been studied. Indeed, it is astonishing that an animal can live in such a frame at all. Considering the pain and discomfort which a single cervical rib, by pressure on the brachial

plexus, can cause in man, I personally have no doubt that pudgy mice are enduring a martyrdom of pain throughout their lives. But as mice do not show suffering in any very obvious way, this cannot at present be proved to the satisfaction of a behaviourist, let alone a philosopher.

DEVELOPMENT

Pudgy embryos down to the 14-day stage can be recognized at a glance by their shortened tails. These are also thinner distally and are pointed at the tip; there is no evidence for any loss of material such as happens in Brachyury ($T/+$). Four litters ranging in age from 14 to 16 days and including twenty-four normal and nine pudgy embryos were stained with methylene blue and cleared in oil of wintergreen. As expected, all the chaotic anomalies of the osseous axial skeleton and of the ribs are preformed in cartilage. Perhaps it would not have been really necessary to make these preparations at all. By no conceivable mechanism could the distortions of the osseous vertebral column have arisen from a normal cartilaginous model. Nor, indeed, could so anomalous a cartilaginous axial skeleton have been formed unless its membranous predecessor was already profoundly out of order.

Normal 13-day embryos show well-defined segmentation (somites) in the distal half of their tails. The tails of 13-day pudgy embryos are not only shortened (though much less than later on), but they also show no clear segmentation distally and only indistinct segments more proximally.

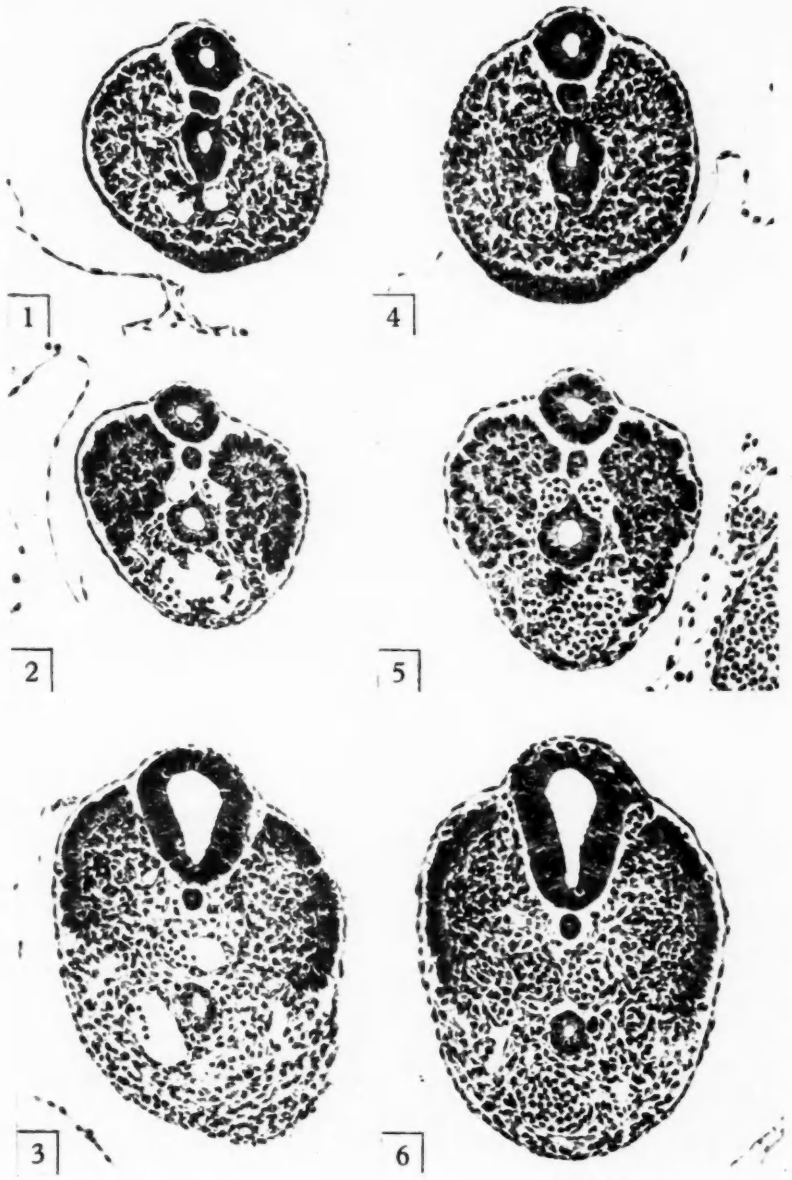
Normal 12-day embryos have somites all along their tails. The tails of pudgy embryos are only slightly shortened, with no segmentation distally and only indistinct segmentation proximally.

In normal 11-day embryos, somites are clearly defined along the tail and the posterior half of the trunk, but they have become indistinct anteriorly. The tails of pudgy embryos are not yet appreciably shortened at this stage. Pudgy embryos can, however, be easily identified by irregularities of their segmentation. As in later stages, segmentation does not reach as far distally as in normal embryos. More proximally, segments are recognizable, but they tend to be somewhat irregular in shape; the intersegmental fissures are rather less well defined than in the normal and they are not always strictly parallel to each other; moreover, the width of adjacent segments is somewhat variable, so that the whole row of somites looks rather untidy. By the same token, pudgy embryos can be distinguished from their normal litter mates without any difficulty in the 10-day and in the 9-day stage. As the whole axial skeleton is similarly affected, there cannot be any doubt that by the same criteria, pudgy embryos could be identified in the 8-day stage and indeed from the onset of somite formation.

For the study of segmentation in sectioned material, two regions have been selected. These are the tail where new segments are formed in 10–12-day embryos, and the lumbo-sacral region where the same phase of development can be studied in the 9-day stage. The two regions behave alike in essentials, but the anomalies are rather more extreme in the tail which, for that reason, may be described first.

Five pudgy embryos and five normal litter-mates ranging in age from 10 to nearly 12 days (C.R.L. 4·3–7·0 mm.) were sectioned transverse to the tail, which at this stage is usually nearly straight. The results are shown diagrammatically in Text-fig. 2 (see also Plate I). In the stages examined, the distal 500 micra or so of the tail still lack segmentation; the paraxial mesoderm forms a fairly homogeneous network of mesenchymatous cells. Part of this region is occupied by the ventral ectodermal ridge (VER) of the tail (Grüneberg, 1957) which resembles the apical ectodermal ridge of the limb buds in structure and may have a similar stimulatory function for the growth of the tail. The VER is shown in transverse sections in Figs. 1 and 4, Plate I, and its extent in proximo-distal direction in Text-fig. 2 (hatched areas). Clearly there is no significant difference between normal and pudgy embryos in this respect. The largest pudgy embryo (No. 1429) has a small VER, but at this stage the structure is in the process of disappearing so that a small difference in stage of development is a sufficient explanation.

In normal embryos, the distal unsegmented tip of the tail is followed proximally by three or four somites (Fig. 2, Plate I) in which the cells are tightly packed without any gaps, and in which the outer layer is like a columnar epithelium in arrangement. The somites are separated from each other by intersegmental fissures which cut through the whole thickness of the somite material. These fissures can be seen in several consecutive sections, and their positions in Text-figs. 2 and 3 represent, as nearly as possible, the centres of the fissures. In the normal embryos, the position of the most distal intersegmental fissure is shown as the end of the somites. Actually, some additional material in the process of forming the next somite is usually present but not shown in the diagrams as its definition is often too unsharp. For this reason, the slight delay in somite formation shown by all five pudgy embryos is probably in reality greater than appears from the diagrams, and I am inclined to regard it as a real difference. Following the three or four somites, there is, in the more cranial segments, an emigration of cells from the ventro-medial aspect of the somites towards the midline; these cells form the sclerotomes and only the outer layer of the original somites remains intact as the dermomyotome. Apart from the fact that somite formation begins a little farther from the tail tip (i.e. later), the general sequence of events is similar in the pudgy embryos, with one important exception. Although closely knit somite tissue is formed, there is a complete absence of intersegmental fissures. The tissue forms one unbroken block which is shorter than the three to four somites in the normals (see also Text-fig. 3). It is only when the somite tissue of the pudgies is cranially transformed into the loose sclerotome tissue that a belated segmentation makes its appearance in the dermomyotomes. This may start fairly promptly (as in embryo 1435) or only after considerable delay (e.g. embryo 1433), and the fissures formed may at first be only rudimentary. For the rest, both the outer shape of the somite block and the inner arrangement of the cells tends to be less regular in pudgy embryos (Fig. 5, Plate I) than in the individual somites of their normal litter-mates. Otherwise, neural tube, notochord, tail gut and the vascular apparatus of the tail seem to be normal.

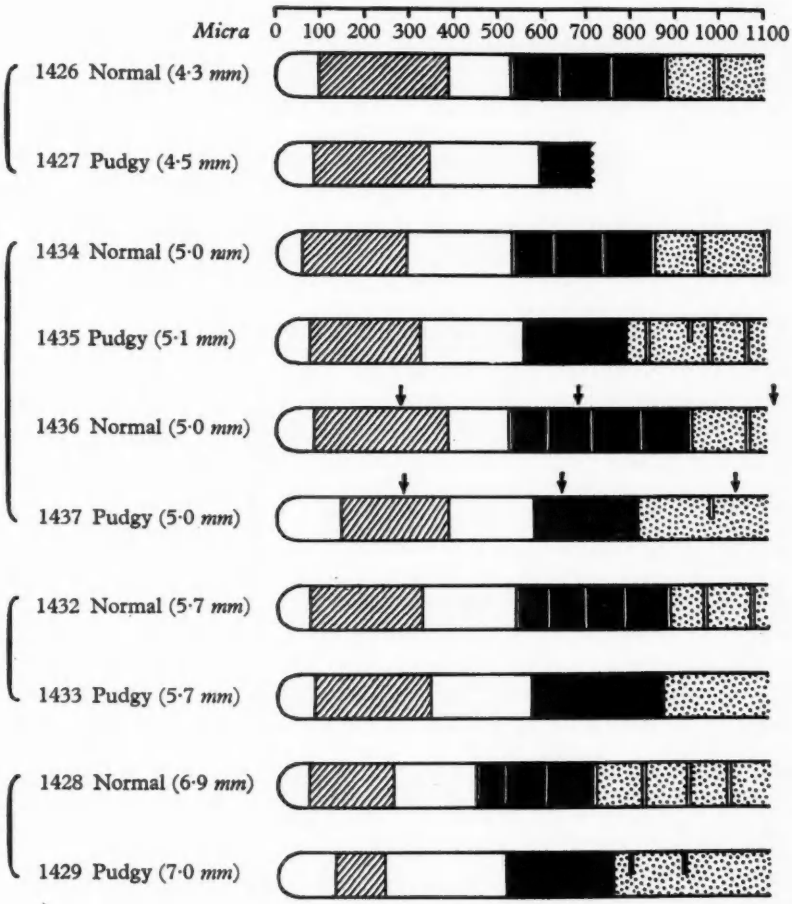


NORMAL PUDGY

Transverse sections through the tails of two 11-day-old embryos. Nos. 1–3 normal No. 1436 (C.R.L. 5·0 mm.), Nos. 4–6 pudgy No. 1437 (C.R.L. 5·0 mm.). In the normal the sections pass through the unsegmented paraxial mesoderm and the ventral ectodermal ridge, through the penultimate somite, and through a dermomyotome respectively; those in the pudgy embryo pass through approximately corresponding levels as indicated by arrows in Text-fig. 2. Bouin fixation; imbedded by Peterfi's method; sections 7·5 micra thick; haematoxylin and eosin. Photographs × 120, enlarged to × 160.

H. GRÜNEBERG (*Facing p.* 388)

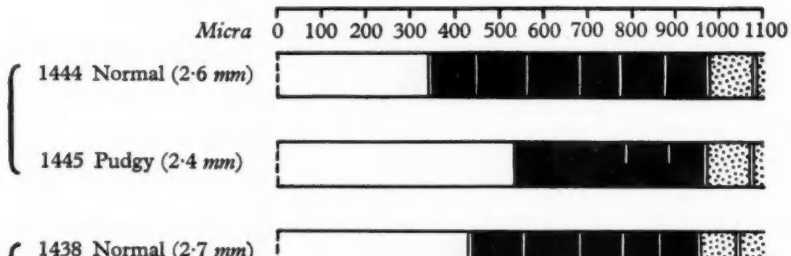
To sum up. In the tail of pudgy embryos, the paraxial mesoderm is formed into solid somite tissue with some delay and in a less orderly fashion. Segmentation does not start until the somite tissue is ventro-medially breaking up to form



Text-fig. 2. Tail diagrams of normal and pudgy embryos aged 10–12 days. Littermates bracketed together. The unsegmented tail tip is shown in white, with the VER hatched. Somites or somite tissue black, sclerotomes stippled. Intersegmental fissures shown in the upper half of a diagram only are rudimentary. Scale in micra starts from the tail tip on the left. In the case of embryo 1427 the proximal part of the tail could not be analysed on account of its curvature. Position of sections in Plate I indicated by arrows.

sclerotomes, and, even then, the fissures separate only consecutive dermomyotomes from each other (and not always completely); the sclerotome tissue, so far as one can see, remains continuous.

At the 9½-day stage (Text-fig. 3) there is hardly any tail bud and the segments in process of formation are the lumbo-sacral ones. As the posterior end of the body is somewhat curved relative to the trunk region, in which segmentation is in progress, the first section which opens the coelom has been used as the starting-point of the scale in micra. The difference between normal and pudgy embryos is similar to that in the tail region. The main difference is that rudimentary inter-



Text-fig. 3. Diagram of segmentation in the process of formation in the lumbo-sacral region of 9½-day-old normal and pudgy embryos. Conventions as in Text-fig. 2, except that the scale in micra starts from the section which first opens the coelom. The position of the third intersegmental fissure from the left in embryo 1444 is by interpolation only as the series was damaged in this neighbourhood.

segmental fissures in the somite block occur in three out of the four pudgies. The anomalies are thus rather less extreme than in the tail, in conformity with the anatomical situation in the adult animal.

In addition to those already described, seven more pudgy embryos, together with seven normal litter-mates, were sectioned in various planes; they included the ages of just under 11 to 12½ days (C.R.L. from 4·7 to 8·3 mm.). They showed, as expected, that the chaotic behaviour of the cartilaginous axial skeleton is pre-

ceded by a similar disorder of the sclerotomes. An additional fact is that the spinal ganglia which, in parasagittal sections of normal embryos, are quite separate from each other, form an almost continuous mass in the pudgies in which their segmental origin is more or less concealed.

DISCUSSION

From the descriptions given above, there cannot be any doubt that the state of the axial skeleton of pudgy mice is basically due to an anomaly of segmentation. The question which immediately arises is whether this is due to anomaly of the unsegmented mesoderm which makes it difficult to subdivide into individual somites, or whether there is some defect in the dynamics of the segmentation process as such, the material to be segmented being normal. This question must remain open at present. I have not been able to discover a consistent difference in the structure of the unsegmented paraxial mesoderm distal to the block of somite material of normal and pudgy mice. Needless to say, other methods might bring such differences to light. On the other hand, both the outer shape and the internal structure of the somite block of pudgy mice is less regular than that of the corresponding somites of normal embryos. However, these irregularities might be either cause or effect of the disturbance of segmentation.

The morphogenesis of the vertebrae of pudgy mice does not seem to be disturbed as such. Wherever there is an opportunity, the regional specificity of the vertebrae asserts itself, and occasionally, such as in the caudal vertebra in Text-fig. 1, a completely normal element may be formed. Evidently, the structural abnormalities are entirely due to the non-separation of the sclerotomes and the consequent scrambling of the chondrogenic material; where circumstances permit, normal or near-normal morphogenesis takes place.

A condition virtually indistinguishable from pudgy is stub (*sb*/*sb*) in the mouse (Dunn & Gluecksohn-Schoenheimer, 1942). There is complete agreement so far as one can judge from the published account. Unfortunately, stub is now extinct so that no direct tests for allelism are possible. For that reason, a distinct name has been used for the present mutant. It is quite possible, if not indeed probable, that pudgy is an allel or a recurrence of stub. Stub was lost before its development had been studied.

Apart from stub in the mouse, by far the most similar inherited condition is short-spine in cattle (Mohr & Wriedt, 1930). The gene is recessive and lethal at or soon after birth. The greatly shortened vertebral column, together with normal head and normal appendicular and girdle skeleton, give the affected calves an elk-like appearance. The anomalies of the telescoped vertebral column, of the ribs and of the sternum are so similar to those of pudgy mice that similar developmental processes are probably responsible for both of them.

It has already been mentioned that the sex-linked mutant Bent-tail in the mouse is probably due to a rather mild disturbance of segmentation (Grüneberg, 1955*b*). Another rather slight involvement of somite formation occurs in the mutant Crooked-tail (*Cd*/+) of the mouse (Matter, 1957) where some of the

somites are reduced in size and deformed, with cell pycnosis in places; these abnormal somites give rise to reduced and deformed blastemata and ultimately cartilaginous and osseous vertebrae. An anomaly of segmentation even more extreme than that of pudgy is found in Rib fusions (*Rf*/*Rf*) recently described by Theiler & Stevens (1960); in these lethal homozygotes, the paraxial mesoderm never forms into solid somite tissue at all; nonetheless, sclerotomes of a kind are formed and a highly telescoped vertebral column with extensive rib fusions is the ultimate result; as in pudgy, the spinal ganglia also tend to coalesce with each other; some involvements of the CNS like exencephaly have not been encountered in pudgy.

In the chick embryo, segregation of somites can be inhibited by various amino-acid antagonists, and particularly by leucine antagonists (Herrmann, 1953; Rothfels, 1954; Herrmann *et al.*, 1955). Recent work by Deuchar (1960) suggests that these substances act by interfering with adenosine triphosphatase activity in the somite mesoderm, and that the activity of this enzyme is one of the main factors in somite formation. The pudgy gene might act in a similar fashion.

SUMMARY

The recessive mutant pudgy in the mouse has a greatly shortened vertebral column with highly irregular fusions between vertebrae and fragments of vertebrae. Ribs and sternum are also involved, but the rest of the skeleton is quite normal. The deformities arise from a defective segmentation. Although the paraxial mesoderm forms somite tissue with an epithelially arranged outer layer, this material either shows only an abortive segmentation into somites or, in the tail, none at all. A belated segmentation into dermomyotomes ultimately takes place, but the sclerotomic material remains continuous and gives rise to erratically abnormal blastemata which then chondrify and ultimately ossify.

I am deeply indebted to Drs W. L. and L. B. Russell, who turned the pudgy mutant over to me for study. The sectioned material and the photomicrographs were made by Miss June Denny, and the drawing by Mr A. J. Lee, to both of whom I wish to express my appreciation. The work was partly supported by a grant from the Rockefeller Foundation which is gratefully acknowledged.

REFERENCES

BERRY, R. J. (1960). Genetical studies on the skeleton of the mouse. XXVI. Pintail. *Genet. Res.* **1**, 439–451.

DEOL, M. S. (1961). Genetical studies on the skeleton of the mouse. XXVIII. Tail-short. *Proc. roy. Soc.* B (in the press).

DEUCHAR, E. M. (1960). Relation between somite segregation rate and ATP-ase activity in early chick embryos. *J. Embryol. exp. Morph.* **8**, 259–267.

DUNN, L. C. & GLUECKSOHN-SCHOENHEIMER, S. (1942). Stub, a new mutation in the mouse with marked effects on the spinal column. *J. Hered.* **33**, 235–239.

GRÜNEBERG, H. (1953). Genetical studies on the skeleton of the mouse. VII. Congenital hydrocephalus. *J. Genet.* **51**, 327–358.

GRÜNEBERG, H. (1954*a*). Genetical studies on the skeleton of the mouse. VIII. Curly-tail. *J. Genet.* **52**, 52–67.

GRÜNEBERG, H. (1954*b*). Genetical studies on the skeleton of the mouse. XII. The development of undulated. *J. Genet.* **52,** 441–455.

GRÜNEBERG, H. (1955*a*). Genetical studies on the skeleton of the mouse. XVI. Tail-kinks. *J. Genet.* **53,** 536–550.

GRÜNEBERG, H. (1955*b*). Genetical studies on the skeleton of the mouse. XVII. Bent-tail. *J. Genet.* **53,** 551–562.

GRÜNEBERG, H. (1957). Genetical studies on the skeleton of the mouse. XIX. Vestigial-tail. *J. Genet.* **55,** 181–194.

GRÜNEBERG, H. (1958*a*). Genetical studies on the skeleton of the mouse. XXII. The development of Danforth's short-tail. *J. Embryol. exp. Morph.* **6,** 124–148.

GRÜNEBERG, H. (1958*b*). Genetical studies on the skeleton of the mouse. XXIII. The development of Brachyury and Anury. *J. Embryol. exp. Morph.* **6,** 424–443.

HERRMANN, H. (1953). Interference of amino-acid analogues with normal embryonic development. *J. Embryol. exp. Morph.* **1,** 291–295.

HERRMANN, H., KONIGSBERG, U. R. & CURRY, M. F. (1955). A comparison of the effects of antagonists of leucine and methionine on the chick embryo. *J. exp. Zool.* **128,** 359–377.

MATTER, H. (1957). Die formale Genese einer vererbten Wirbelsäulenmissbildung am Beispiel der Mutante Crooked-tail der Maus. *Rev. suisse Zool.* **64,** 1–38.

MOHR, O. L. & WRIEDT, C. (1930). Short spine, a new recessive lethal in cattle; with a comparison of the skeletal deformities in short-spine and in amputated calves. *J. Genet.* **22,** 279–297.

ROTHFELS, U. (1954). The effects of some amino acid analogues on the development of the chick embryo *in vitro*. *J. exp. Zool.* **125,** 17–37.

THEILER, K. & STEVENS, L. C. (1960). The development of rib fusions, a mutation in the house mouse. *Amer. J. Anat.* **106,** 171–183.

Genet. Res., Camb. (1961), **2**, *pp.* 394–405
With 3 text-figures
Printed in Great Britain

The genetics of fimbriation in *Escherichia coli*

By G. A. MACCACARO and W. HAYES

*Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, W.*12

(*Received* 22 *April* 1961)

INTRODUCTION

Fimbriae are filamentous, non-flagellar appendages present at the surface of a number of Gram-negative *Eubacteriales* (Anderson, 1949; Houwink & Van Iterson, 1950; Brinton, Buzzell & Lauffer, 1954; Duguid, Smith, Dempster & Edmunds, 1955; Constable, 1956; Duguid & Gillies, 1957, 1958) including *Escherichia coli K*-12 (Maccacaro, 1955; Maccacaro, Colombo & DiNardo, 1959; Brinton, 1959). This paper reports a genetic analysis of this character, which is of interest not only from a morphological point of view, but also because it affects some surface properties and basic physiological traits of the bacterial cell.

Types of fimbriation

Within the same species two main types of strain have been described (Brinton *et al.*, 1954; Duguid *et al.*, 1955; Maccacaro *et al.*, 1959; Maccacaro & Turri, 1959): (*a*) those which either possess fimbriae or can develop them under suitable environmental conditions, and (*b*) strains which lack fimbriae irrespective of the environmental conditions. The notations 'Fim$^+$' and 'Fim$^-$' have been applied to these two types; within a Fim$^+$ strain, the notation 'Fim$^+$' indicates expression, and 'Fim$^{(+)}$' lack of expression, of the character. A Fim$^{(+)}$ strain usually becomes Fim$^+$ if serially subcultured in nutrient broth, but reverts to the Fim$^{(+)}$ state after repeated subculture on nutrient agar. These Fim$^+ \rightleftharpoons$ Fim$^{(+)}$ conversions are always reversible and are not reflected in changes in colonial morphology. This is true for practically all species and genera which have so far been tested.

A rather different picture was presented by Brinton (1959), who reported that, in some strains of *E. coli B*, fimbriated cells (P$^+$) give rise to morphologically distinctive colonies, but mutate irreversibly to a non-fimbriated type (P$^-$) at a high rate (4×10^{-4}/cell/generation). The gap between these conflicting pictures was filled by the discovery that Fim$^+$ strains of *E. coli* K-12, if plated on nutrient agar after a long series of subcultures in fluid medium, give rise, among a majority of normal colonies, to a few small, smooth, compact colonies which are entirely composed of fimbriated cells. Occasionally some of these colonies produce a large rough outgrowth consisting of non-fimbriated cells. The former, colonially distinctive, type is insensitive to those environmental factors which controlled the Fim$^+ \rightleftharpoons$ Fim$^{(+)}$ conversion of the parental strain; the latter type is irreversibly non-fimbriated. These two types clearly correspond to Brinton's P$^+$ and P$^-$ types.

We have adopted the notation Fimσ^+ to indicate the stably fimbriated mutants which arise from Fim$^+$ strains, and the notation Fimσ^- to indicate the irreversibly non-fimbriated mutants which arise from Fimσ^+ strains. Our present knowledge about types of fimbriation is summarized in Fig. 1.

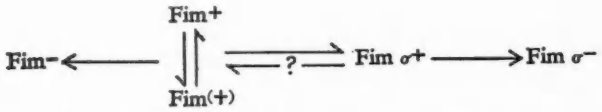


Fig. 1. Types of fimbriation and non-fimbriation in *Escherichia coli* K-12, and the relationship between them.

Detection of fimbriae and correlated properties

Fimbriae cannot be seen by light microscopy but are easily detectable by electron-microscopy, especially in shadowed preparations (Anderson, 1949; Houwink & Van Iterson, 1950; Brinton *et al.*, 1954; Duguid *et al.*, 1955; Constable, 1956; Duguid & Gillies, 1957, 1958; Maccacaro, 1955). Their presence on individual cells is marked by a sharp reduction in electrokinetic mobility (Brinton *et al.*, 1954; Maccacaro & Turri, 1959; Brinton, 1959). Fimbriated cells of both Fim$^+$ and Fimσ^+ types form a tenacious pellicle in broth culture and agglutinate red blood cells of the guinea-pig and other animals (Duguid *et al.*, 1955; Maccacaro *et al.*, 1959); in the case of Fimσ^+, however, this agglutination is more or less suppressed in the presence of peptone. Fimbriated cells are also agglutinated by a wide variety of dipolar molecules (dicarboxylic acids, diamines and monoamino-monocarboxylic acids) (Maccacaro & Dettori, 1960). In addition to the effect of environmental factors such as culture media and certain chemical agents (Table 1) on the expression of fimbriation, certain Fim types differ in their oxidative and glycolytic activity (Maccacaro & Dettori, 1959). When all these properties are considered together, it is possible to distinguish five types of cell with respect to fimbriation, of which two are fimbriated (Fim$^+$ and Fimσ^+) and three non-fimbriated (Fim$^-$, Fimσ^-, and Fim$^{(+)}$). These distinguishing properties are set out, with references, in Table 1.

Genetic mapping of the fim *locus*

A morphological character whose expression can be so easily elicited or repressed (Fim$^+ \rightleftharpoons$ Fim$^{(+)}$), as well as stabilized (Fimσ^+) and then irreversibly lost (Fimσ^-), seems to offer an unusual opportunity for genetic analysis of the control of bacterial morphogenesis. In the experiments reported here, haemagglutination of guinea-pig R.B.C. was the principal test used to discriminate between fimbriated and non-fimbriated recombinant types; Fim$^+$ and Fimσ^+ recombinants were distinguished by inhibition of this agglutination by peptone, as well as by colonial morphology.

Table 1. *Distinction between different types of fimbriation and non-fimbriation in* Escherichia coli *K-12*

Expression of fimbriation	Types of fimbriation: Fim$^-$	Fim$^{(+)}$	Fim$^+$	Fimσ^+	Fimσ^-	References
Presence of fimbriae (electron microscopy)	– (not reversible)	– (reversible)	+	+	– (not reversible)	Anderson, 1949 Maccacaro, 1955
Sensitivity of fimbriation to cultural conditions	–	+	+	–	–	Duguid *et al.*, 1955 Maccacaro & Turri, 1959
Inhibition of expression of fimbriation by ethanol* & phenol†			+	–		
Agglutination of red blood cells	–	–	+	+ (Inhibited by peptone)	–	Duguid *et al.*, 1955 Maccacaro *et al.*, 1959
Agglutination by dipolar molecules	–	–	+	+	–	Maccacaro & Dettori, 1960
Electrophoretic mobility	fast	fast	slow	slow	fast	Brinton *et al.*, 1954 Maccacaro & Turri, 1959 Brinton, 1959
Q_{O_2}‡ *a*	26	95	90			Maccacaro & Dettori, 1959
Q_{O_2}‡ *b*		86	83	85	88	
Q_{CO_2}§ *a*	205	190	90			Maccacaro & Dettori, 1959
Q_{CO_2}§ *b*		172	69	85	78	

* = 4 per cent ethanol in nutrient broth.
† = 0·1 per cent phenol in nutrient broth.
‡ = substrate: sodium fumarate M/60.
§ = substrate: glucose M/240.
a = recombinants α_4 and β_1 (see Maccacaro & Dettori, 1959).
b = strain 58-161.

Earlier investigations (Maccacaro *et al.*, 1959), prior to the discovery of the σ types, had led to the following conclusions:

(*a*) In Fim$^+$ F$^+$ × Fim$^-$F$^-$ crosses, where the F$^+$ donor loci *thr*$^+$ and *leu*$^+$ (ability to synthesize threonine and leucine) are selected, 70 to 80% of the prototrophic recombinants are fimbriated.

(*b*) The locus controlling fimbriation (*fim*) is rather closely linked to the *thr–leu* region.

(*c*) Clearcut mapping of the *fim* locus is perturbed by a marked, direct correlation between the length of Fim$^+$ donor chromosome inherited by recombinant cells and the frequency of fimbriation among them.

(*d*) The rapidity with which fimbriation is expressed in Fim$^+$ recombinants is correlated with the degree of expression of the fimbriated parent.

Findings (*c*) and (*d*) suggest that Fim$^+$ inheritance might depend on a cytoplasmic as well as on a chromosomal contribution from the Fim$^+$ donor parent, but the possibility of negative interference was also considered as a possible explanation of finding (*c*). Furthermore, since F$^+$ × F$^-$ crosses were used, it was possible that the anomalies of recombination were due to heterogeneity among the F$^+$ chromosome fragments transferred to the zygotes.

EXPERIMENTAL METHODS AND RESULTS

Genetic analysis by means of Hfr × F$^-$ crosses

The validity and precision of genetic analysis employing Hfr × F$^-$ crosses is now well established (Wollman & Jacob, 1959). We therefore turned to the study of inheritance of fimbriation by means of such crosses. Strain Hfr*H* (Hayes, 1953)

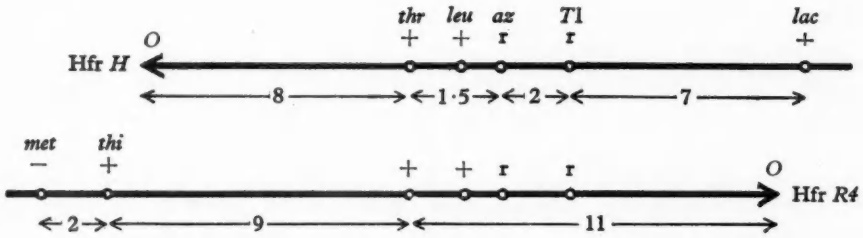


Fig. 2. Chromosome transfer by the Hfr strains *H* and *R4*. The diagram shows the leading chromosomal extremity, *O*, and the direction of chromosome transfer (indicated by arrow) by each strain. The distances between the relevant loci, in terms of times of transfer (minutes) in broth at 37°, are roughly to scale. Both strains are derived from the same *F*$^+$ strain and are identical in their nutritional requirements and other characters.

met = methionine, *thi* = thiamine, *thr* = threonine, *leu* = leucine } synthesis

az^r = sodium azide resistance
T1^r = phage T1 resistance
lac$^+$ = lactose fermentation

Note: Hfr*H* transfers the segment *thr–lac* in less time than Hfr*R4* takes to transfer the shorter segment *O–thr*. This is accounted for by the time required by Hfr*R4* to *initiate* transfer (for other examples, see Taylor & Adelberg, 1960).

was used as Fim$^+$ donor; its relevant loci, the order of their arrangement on the chromosome and the approximate distances between them in terms of time of transfer in broth at 37° C. (Wollman, Jacob & Hayes, 1956), are given in Fig. 2. The F$^-$ strain (W945) was Fim$^-$ and complementary to Hfr*H* in all these characters. The segregation of unselected markers in prototrophic recombinants selected for *thr*$^+$ and *leu*$^+$ inheritance from the Hfr parent was orthodox, but the distribution of *fim*$^+$ within the different recombinant classes revealed the same anomaly observed in the Fim$^+$F$^+$ × Fim$^-$F$^-$ crosses in an even more striking way (see Table

2A; also Maccacaro & Hayes, 1961). It therefore seemed that the *fim* locus could not be precisely located by means of linkage data. However, the unique features of conjugation in *E. coli* (Wollman, Jacob & Hayes, 1956) enable the position of a locus to be defined on a time scale which can be converted into a chromosome map. By periodic interruption of the mating process between conjugating cells it is possible to determine with accuracy the order and time of entry of donor genes into the recipient cells and, therefore, their sequence and distance from one another on the donor chromosome.

The Fim$^-$ streptomycin-resistant (S^r) strain W945 was used as recipient in interrupted mating experiments with two different Fim$^+$ streptomycin-sensitive (S^s) Hfr strains, *H* and *R4*. These Hfr donor strains are both derived from the same F$^+$ strain (58-161) and have the same genotype, but they differ in the position of the leading locus (*O*) and in the direction of entry of the chromosome into the recipient cells as shown in Fig. 2. The two crosses were carried out in the same way. Young broth cultures of the parental strains were mixed and kept in gentle

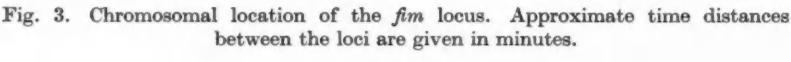


Fig. 3. Chromosomal location of the *fim* locus. Approximate time distances between the loci are given in minutes.

agitation at 37°. Samples of the mixture were removed at one-minute intervals, supplemented with streptomycin (100 μg./ml) and vigorously shaken in a blendor. The blended mixture was then diluted 10^{-3} and small loopfuls were plated on minimal agar + streptomycin to score for the appearance of *thr*$^+$*leu*$^+$ recombinants; these recombinants were later scored for inheritance of *fim*$^+$. Similar loopfuls were also inoculated into test-tubes containing nutrient broth + streptomycin to score for the appearance of *fim*$^+$ recombinants which were later tested for inheritance of *thr*$^+$ and *leu*$^+$; the pellicle-forming ability of Fim$^+$ cells confers upon them such a selective advantage that they overgrow Fim$^-$ cells even if present as a minute minority in the inoculum. However, the presence of fimbriae was always confirmed by haemagglutination tests.

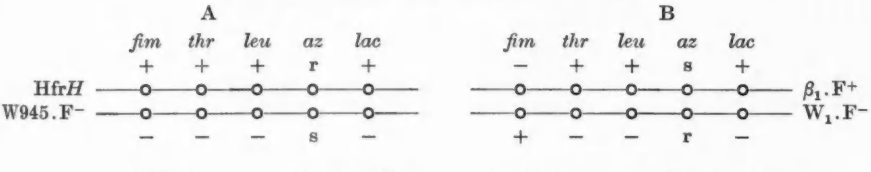
Results showed that when Hfr*H* is the donor, fimbriated recombinants begin to appear several minutes before *thr*$^+$*leu*$^+$ recombinants are detectable; conversely, when Hfr*R4* is the donor, the early *thr*$^+$*leu*$^+$ recombinants are non-fimbriated. More precisely, *fim*$^+$ enters recipient cells 2–3 minutes ahead of *thr*$^+$ in the Hfr*H* × W945 cross but 2–3 minutes after the same marker in the Hfr*R4* × W945 cross. These consistent results show that *fim* is located close to *thr*, at about one-quarter of its distance from *thi*, as shown in Fig. 3. This location for *fim* has been confirmed by the results of other crosses involving different donor and recipient strains and other selective and unselected markers. It accounts for previous difficulties in the mapping of *fim* which is thus the only known marker situated between the leading locus of the Hfr*H* chromsome and the *thr—leu* region.

Anomalies of fim *segregation*

On the basis of its location proximal to *thr* on the Hfr*H* chromosome, the exclusion of the *fim*$^+$ locus from the 20–30% of *thr*$^+$*leu*$^+$ recombinants could be interpreted formally in terms of the ratio of the probability of recombination in the short region *fim–thr* (2–3 minutes) to that of recombination in the much longer region *O–fim* (6 minutes). What the location of *fim* failed to explain was the fact (Table 2A) that the inheritance of *fim*$^+$ increases proportionately as the inherited length of chromosome distal to the selective markers *thr*$^+$*leu*$^+$ increases. The possibility that this anomaly might reflect peculiarities of the Fim$^+$ character and its expression, rather than location of the locus, was challenged by the analysis of reversed crosses between a Fim$^-$ donor and a Fim$^+$ recipient strain. Since a Fim$^-$ Hfr strain was not available, a Fim$^-$F$^+$ recombinant strain β_1 (Maccacaro & Dettori, 1959), derived from a cross between strains 58-161 Fim$^+$F$^+$ and W945 Fim$^-$F$^-$, was used as donor: the recipient strain was W_1 F$^-$ (see Table 2B for details) and *thr*$^+$*leu*$^+$ recombinants were selected. Table 2 compares the results

Table 2. *Percentage inheritance of the loci* fim$^+$ *and* fim$^-$ *among recombinant classes inheriting varying lengths of the donor chromosome to the right of the selective markers* thr$^+$leu$^+$

Distances between loci are not drawn to scale



thr$^+$ *leu*$^+$ recombinants	Per cent *fim*$^+$ inheritance	*thr*$^+$ *leu*$^+$ recombinants	Per cent *fim*$^+$ inheritance
az^s *lac*$^-$	50	*az*^{r} *lac*$^-$	47
az^r *lac*$^-$	68	*az*^{s} *lac*$^-$	29
az^r *lac*$^+$	89	*az*^{s} *lac*$^+$	15

of this cross (B) with those of the cross Hfr*H* × W945 (A). The same correlation is found between the frequency of inheritance of the *fim* locus and that of increasing lengths of chromosome to the right of *thr*$^+$*leu*$^+$, irrespective of whether the donor parent is Fim$^+$ or Fim$^-$. We therefore favour the view that the correlation is a consequence of the position of the locus rather than of the nature of its function, and should be interpreted as reflecting negative interference over large regions of the K-12 chromosome. Further data and a more expanded discussion of this problem are presented in another paper (Maccacaro & Hayes, 1961).

Transducibility of fim

Quantitative study of the genetics of fimbriation is limited by inability to select for, or even to recognize, colonies of Fim$^+$ cells within Fim$^-$ populations and vice versa. The only exception is the distinctive appearance of Fimσ^+ colonies

which can be distinguished from those of Fim$^+$, Fim$^-$ and Fimσ^- cells. Various attempts to devise a selective or differential technique based on the different oxidative ability of Fim$^+$ and Fim$^-$ cultures (Table 1) were not successful. This limitation becomes particularly stringent in transduction experiments, especially since the distance of the *fim* locus from the nearest selective marker (*thr*$^+$) is so great as to preclude joint transduction. Nevertheless, it has been possible to demonstrate the transducibility of fimbriation into non-fimbriated cells by making use of the great selective advantage conferred on fimbriated cells by their ability to form a tenacious pellicle on the surface of broth cultures (see above).

The following technique was used. Streptomycin-sensitive Fim$^+$ and Fimσ^+ variants of the same strain (58-161 F$^+$) were used as donors. Strain W945.S^r(Fim$^-$) was the usual recipient, but in some experiments a Fimσ^- variant of 58-161.F$^-$ was employed. Phage Plkc (kindly supplied by Dr S. W. Glover) was the transducing agent. Phage lysates (10^{10}–10^{11} plaque-forming units/ml.) from each of the two donors were added to young broth cultures of the recipient at multiplicities ranging from 10 to 10^{-4}. These adsorption mixtures were kept at room temperature for 90 minutes. One-millilitre samples were then distributed into a series of bottles containing 100 ml. broth + streptomycin, thus permitting at least six to eight generations for the expression of fimbriation by transduced cells. These broth cultures were incubated at 37° for 4 days, and then scored for pellicle formation and haemagglutinating activity. Samples were also subcultured to fresh broth to allow further time for the expression of fimbriation, and plated on nutrient agar for examination of single colonies.

The results of a number of such experiments support the following general conclusions.

(1) Fimbriated transductants inherit the Fim status (i.e. Fim$^+$ or Fimσ^+) of the donor parent, irrespective of whether the recipient is Fim$^-$ or Fimσ^-.

(2) The efficiency of transduction of fimbriation is higher with Fimσ^+ than with Fim$^+$ donors, with respect both to frequency and to the time required for pellicle formation. This was apparent despite the crudity of the selective method which blurs the determination of absolute transduction frequencies. On the other hand, substitution of Fim$^-$ by Fimσ^- as recipient did not alter the efficiency of transduction.

(3) Transduction of either the Fim$^+$ or the Fimσ^+ state is unlinked to that of any other marker tested. Similarly, transductants selected for *thr*$^+$ or *thr*$^+$*leu*$^+$ inheritance, from fimbriated donors, are non-fimbriated. It is worth emphasizing that more subtle features than are yet apparent in the transduction of fimbriation may well be masked by the coarseness of the present selective technique.

The relationship between Fim$^+$ and Fimσ^+ types

The main physiological feature characterizing the Fimσ^+ type is the stability of its fimbriation to environmental conditions, such as repeated subculture on solid medium or exposure to phenol or ethanol, which readily repress expression

in Fim$^+$ strains. This difference might be controlled, at the genetic level, by the interaction of a second chromosomal gene or of a cytoplasmic factor, or by stabilization of an episome (Jacob, Schaeffer & Wollman, 1960) in its chromosomal state. Fim$^+$ and Fimσ^+ variants of the donor strain 58-161 F$^+$ were therefore crossed, in parallel, with the Fim$^-$F$^-$ recipient strain W945.S^r in an attempt to analyse the situation. It was found that:

(1) Recombinants from Fim$^+$ × Fim$^-$ crosses are either Fim$^+$ or non-fimbriated.

(2) The frequency of Fim$^+$ recombinants from such crosses remains the same whether the fimbriation of the donor parent is expressed (Fim$^+$) or not (Fim$^{(+)}$).

(3) Recombinants from Fimσ^+ × Fim$^-$ crosses are either Fimσ^+ or non-fimbriated.

(4) The two types of cross do not differ significantly in the overall percentage of fimbriated recombinants.

These findings suggest that the Fimσ^+, as well as the Fim$^+$, state behaves as an integrated chromosomal function and that, if the Fim$^+$ → Fimσ^+ variation depends on mutation at a second, specific locus, this locus is likely to be very close to *fim*. These findings conform to those of the transduction experiments, but it is worth noting here that a few Fim$^+$ transductants were occasionally recovered from Fimσ^+ × Fim$^-$ transductions. These may represent exceptional Fimσ^+ → Fim$^+$ reversions, but the possibility that they arose from rare crossovers between *fim* and a very closely linked hypothetical σ locus cannot be excluded. It is known that closely linked loci are more often separable by recombination in crosses mediated by transduction than in conjugal crosses (see Maccacaro & Hayes, 1961). This view is supported by other findings reported in the next section.

The relationship between Fim$^-$ and Fimσ^- types

The two stably non-fimbriated types are distinguishable, at the biochemical level, by the greater oxidative activity of the Fimσ^- type and the greater glycolytic activity of the Fim$^-$ type (Table 1). We therefore tried to establish a genetic difference between them. Since, of the only two strains available for comparison, one (Fimσ^-; 58-161) required methionine (*met. thr$^+$leu$^+$*) and was S^s and F$^+$, while the other (Fim$^-$; W945) was *met$^+$thr.leu*, S^r and F$^-$, it was first necessary to make these strains identical except for their non-fimbriated status. This was done as follows:

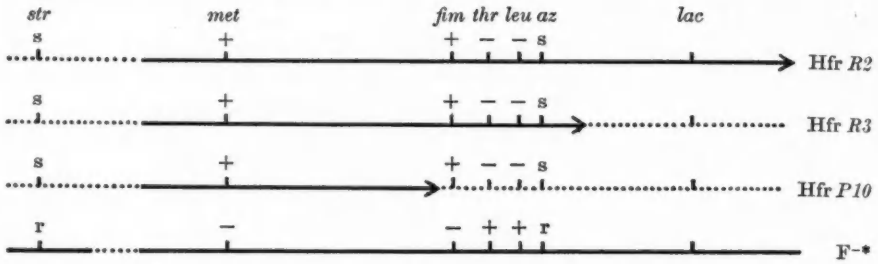
(1) The Fimσ^- strain. An S^r mutant was selected and then converted to F$^-$ by acriflavine treatment (Hirota & Iijima, 1957) (= *met.thr$^+$leu$^+$*.S^r.F$^-$).

(2) The Fim$^-$ strain was first reverted to *thr$^+$leu$^+$* by a two-step selection of back-mutants; the absence of auxotrophic recombinants in crosses with other *thr$^+$leu$^+$* strains showed that these reversions were not due to suppressor mutations at other loci. The requirement for methionine was then introduced by means of interrupted mating with Hfr*R*1 which transfers *met* as an early marker (= *met. thr$^+$leu$^+$*.S^r.F$^-$).

The Fim⁻ and Fimσ⁻ strains so obtained were used as recipients in crosses with three different Fim⁺ *met⁺thr.leu* Hfr strains (*P*10, *R*2 and *R*3) which differed only in the position of their leading loci (Table 3). Selection was made for *met⁺thr⁺leu⁺* recombinants which were purified by single colony isolation and then scored for Fim status. Since the formation of these recombinants requires recombination in the *met–thr* region, which includes the *fim* locus, it was hoped that the resolution

Table 3. *Genetic crosses distinguishing the two non-fimbriated types, Fim⁻ and Fimσ⁻*

On the chromosome maps of the Hfr strains, the continuous line indicates the segment of chromosome transferred to the zygotes, and the arrow the direction of transfer



* For specifications of recipient strains used, see text.

Fim⁺ Hfr donor	Per cent Fim types among *met⁺ thr⁺ leu⁺* recombinants: Fim⁻ recipient			Fimσ⁻ recipient		
	Fim⁺	Fimσ⁺	non-fimbriated	Fim⁺	Fimσ⁺	non-fimbriated
*R*2	11	0 (180)†	89	18	4 (163)	78
*R*3	0	0 (241)	100	6	12 (123)	82
*P*10	0	0 (75)	100	0	0 (75)	100

† Figures in parentheses = total number recombinants tested from two or more separate crosses.

of analysis in the *fim* region might be increased. Details of these crosses and of their outcome are presented in Table 3. The appearance of an appreciable number of Fimσ⁺ recombinants from the crosses between Fimσ⁻ and the two Fim⁺ Hfr strains, *R*2 and *R*3, indicates rather strongly that Fimσ⁻ cells retain some part of the genetic determinant of the Fimσ⁺ state which is absent from Fim⁻ cells. The results of the crosses with strain Hfr*R*3 accentuate still more the genetic difference between Fim⁻ and Fimσ⁻, but raise the problem (which we have so far been

unable to solve) of why the Fim$^+$ character is not inherited at all by recombinants issuing from the Fim$^-$ recipient. However, this genetic difference was not apparent when the two non-fimbriated types (the Fim$^-$F$^+$ recombinant β_1—see above—and the Fimσ^- strain 58-161 F$^+$) were used as donors in crosses with a Fim$^+$F$^-$ recipient strain (W_1—see Table 2). Both crosses yielded the same proportions of Fim$^+$ and non-fimbriated recombinants, but no Fimσ^+ progeny: it is not known whether the non-fimbriated recombinants obtained from the Fimσ^- donor were Fim$^-$ or Fimσ^-.

DISCUSSION

Speculative models for fimbriation

The physiological and genetic differences between the two non-fimbriated types, Fim$^-$ and Fimσ^- (Tables 1 and 3), together support the idea that fimbriation is a function of the activity of at least two genetic determinants. If we call these determinants *A* and *B* and designate mutations in them, leading to loss or alteration of function, *a* and *b* respectively, we can construct three theoretical models for the determination of fimbriation (Table 4). In all models *A* and *B* would have to

Table 4. *Possible models of the genetic control of fimbriation*

At least two determinants *A* and *B*, are assumed

Model 1: *A* and *B* are chromosomal genes; *A* controls synthesis of fimbriae and *B* the type of fimbriation.

Model 2: *A* and *B* are chromosomal genes, together controlling synthesis of fimbriae; type of fimbriation is independently controlled.

Model 3: *A* is a chromosomal gene and *B* an episome; type of fimbriation is a function of the location of *B*.

Fimbriation status	Model 1	Model 2	Model 3
Fimσ^+	*AB*	*AB*	*AB*
Fim$^+$	*Ab*	*AB*	$A \rightleftharpoons B$
Fimσ^-	*aB*	*Ab*	*A*
Fim$^-$	*ab*	*aB*	*a*

be very closely linked in order to account for the mapping data which show that the loci for both types of fimbriation and of non-fimbriation are located within the same small region, as well as for the conformity of recombinants to the donor fimbriated type, irrespective of the type of the non-fimbriated recipient, in crosses mediated both by transduction and conjugation.

Model 1 supposes that *A* alone determines production of fimbriae, while the function of *B* is control of the type of fimbriation. This model fails to account for the irreversibility of the Fim$\sigma^+ \rightarrow$ Fimσ^- mutation and for the difference in oxidative capacity between Fim$^-$ and Fimσ^-; it is, of course, possible that the reduced Q_{O_2} of the Fim$^-$ strain W945 is due to mutation involving an oxidative function which only indirectly represses synthesis of fimbriae, but the location of the mutational site within the *fim* region makes this unlikely. In model 2, both

A and *B* are necessary for production of fimbriae, the Fim$^+$ → Fimσ^+ variation being controlled independently; the genetic data require, however, that the site of this independent control be closely linked to the *AB* system. This model explains the oxidative defect of the Fim$^-$ type which alone has a mutation in *A*, but not the irreversibility of the Fim$^-$ and Fimσ^- mutations, nor the fact that fimbriated recombinants have never been isolated from crosses, of reversed *F* polarity, between the two non-fimbriated types (i.e. by $Ab \times aB$) even when the sensitive method of selecting for fimbriated cells by pellicle formation in broth was used. Model 3 supposes that determinant *B* is an episome which, in co-operation with the chromosomal gene *A*, determines synthesis of fimbriae; its permanent fixation to the chromosome could account for stabilization of the character in Fimσ^+ cells while its spontaneous loss would explain the peculiar ability of Fimσ^+ cells to mutate irreversibly, at a relatively high rate, to the non-fimbriated Fimσ^- state. If maintainance of episome *B* depended on a functional gene *A*, then mutation in *A* would result in loss of *B* and thus also explain the irreversibility of the mutation to Fim$^-$. Certain Fim$^+$ cultures have shown some evidence of a diphasic variation between the Fim$^+$ and Fimσ^+ states, which could correspond to alternation of an episome between its non-chromosomal and chromosomal locations. However, there are two lines of evidence against this plausible model which we initially favoured. The first is that Fimσ^-.F$^+$ × Fim$^+$.F$^-$ crosses yield 20–30 % of non-fimbriated recombinants, while model 3 predicts that all recombinants should be Fim$^+$. Secondly, all attempts to transfer fimbriation from Fim$^+$ to Fimσ^- cells, either by contact or conjugal infection which excluded transfer of the *fim* locus (see Hfr.*P*10 cross, Table 3), were entirely unsuccessful. These experiments also preclude models involving purely cytoplasmic determinants (plasmids). The one thing that is clear is that the physiological and genetic mechanisms of the control of fimbriation are likely to prove as complicated as Fig. 1 would suggest. It is important to point out, however, that we have so far examined only one strain of each of the types Fimσ^+, Fimσ^- and Fim$^-$; it is thus possible that one or more of these may have resulted from a deletion or be otherwise atypical.

SUMMARY

(1) Fimbriated cells of *E. coli K*-12 are of two types. In the Fim$^+$ type the expression of fimbriation is susceptible to reversible environmental suppression. This type gives rise to environment-stable mutants termed Fimσ^+.

(2) Fimbriated cells can yield two types of non-fimbriated mutant, Fim$^-$ and Fimσ^-, the latter arising from populations of Fimσ^+ cells. Neither type reverts to the fimbriated state.

(3) Both types of fimbriated and non-fimbriated cells can be distinguished by physiological and genetic criteria.

(4) Fimbriation of both types can be transferred to non-fimbriated cells of both types, and segregates among recombinants, in crosses mediated either by transduction or sexual conjugation.

(5) The genetic control of fimbriation involves at least two determinants, for one of which a chromosomal location (*fim*) has been mapped.

(6) Certain anomalies of *fim* segregation are interpreted in terms of negative interference over relatively large regions of the bacterial chromosome.

We are much indebted to Drs F. Jacob and E. L. Wollman for kindly supplying us with the Hfr strain *P*10, and to Dr P. Reeves for the gift of his Hfr strains *R*1, *R*2, *R*3 and *R*4.

One of us (G. A. M.) gratefully acknowledges the financial support given him by the Italian Consiglio Nazionale delle Ricerche.

REFERENCES

ANDERSON, T. F. (1949). In *The Nature of the Bacterial Surface* (eds. A. A. Miles & N. W. Pirie). Oxford: Blackwell. *Symp. Soc. gen. Microbiol.* **1**, 76–95.

BRINTON, C. C. (1959). Non-flagellar appendages of bacteria. *Nature, Lond.*, **183**, 782–786.

BRINTON, C. C., BUZZELL, A. & LAUFFER, M. A. (1954). Electrophoresis and phage susceptibility studies on a filament-producing variant of the *E. coli* B bacterium. *Biochim. Biophys. Acta*, **15**, 533–542.

CONSTABLE, F. D. (1956). Fimbriae and haemagglutinating activity in strains of *Bacterium cloacae*. *J. Path. Bact.* **72**, 133–136.

DUGUID, J. P. & GILLIES, R. R. (1957). Fimbriae and adhesive properties in dysentery bacilli. *J. Path. Bact.* **74**, 397–411.

DUGUID, J. P. & GILLIES, R. R. (1958). Fimbriae and haemagglutinating activity in *Salmonella, Klebsiella, Proteus* and *Chromobacterium*. *J. Path. Bact.* **75**, 519–520.

DUGUID, J. P., SMITH, I. W., DEMPSTER, G. & EDMUNDS, P. N. (1955). Non-flagellar filamentous appendages ('fimbriae') and haemagglutinating activity in *Bacterium coli*. *J. Path. Bact.* **70**, 335–348.

HAYES, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 75–93

HIROTA, Y. & IIJIMA, T. (1957). Acriflavine as an effective agent for eliminating *F* factor in *Escherichia coli* K-12. *Nature, Lond.*, **180**, 655–656.

HOUWINK, A. L. & VAN ITERSON, W. (1950). Electron microscopical observations on bacterial cytology: II. A study on flagellation. *Biochim. Biophys. Acta*, **5**, 10–44.

JACOB, F., SCHAEFFER, P. & WOLLMAN, E. L. (1960). Episomic elements in bacteria. In *Microbial Genetics* (eds. Hayes, W. & Clowes, R. C.). Cambridge University Press. *Symp. Soc. gen. Microbiol.* **10**, 67–91.

MACCACARO, G. A. (1955). Cell surface and fertility in *Escherichia coli*. *Nature, Lond.*, **176**, 125–126.

MACCACARO, G. A., COLOMBO, C. & DINARDO, A. (1959). Studi sulle fimbrie batteriche: I. Lo studio genetico delle fimbrie. *Gior. Microbiol.* **7**, 1–20.

MACCACARO, G. A. & DETTORI, R. (1959). Studi sulle fimbrie batteriche: IV. Metabolismo ossidativo e fermentativo in cellule fimbriate e sfimbriate. *Gior. Microbiol.* **7**, 52–68.

MACCACARO, G. A. & DETTORI, R. (1960). *Gior. Microbiol.* **8**, 65.

MACCACARO, G. A. & HAYES, W. (1961). Pairing interaction as a basis for negative interference. *Genet. Res.* **2**, 406–413.

MACCACARO, G. A. & TURRI, M. (1959). Studi sulle fimbrie batteriche: II. Osservazioni microelettroforetiche. *Gior. Microbiol.* **7**, 21–36.

TAYLOR, A. L. & ADELBERG, E. A. (1960). Linkage analysis with very high frequency males of *Escherichia coli*. *Genetics*, **45**, 1233–1243.

WOLLMAN, E. L. & JACOB, F. (1959). *La Sexualité des Bactéries*. Paris: Masson & Cie.

WOLLMAN, E. L., JACOB, F. & HAYES, W. (1956). Conjugation and genetic recombination in *Escherichia coli* K-12. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 141–162.

Genet. Res., Camb. (1961), **2**, *pp.* 406–413
With 1 *text-figure*
Printed in Great Britain

Pairing interaction as a basis for negative interference

By G. A. MACCACARO and W. HAYES

*Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London, W.*12

(*Received* 22 *April* 1961)

The recurring problem of negative interference in conjugal crosses of *Escherichia coli* K-12 (Rothfels, 1952; Cavalli-Sforza & Jinks, 1956; Wollman, Jacob & Hayes, 1956) has been periodically overshadowed by new discoveries concerning the mechanism of genetic transfer in this organism, such as unidirectional and partial transfer (Hayes, 1953; Wollman *et al.*, 1956) and the genetic heterogeneity of zygotes formed in $F^+ \times F^-$ crosses (Jacob & Wollman, 1957), which have led to reappraisal of the interpretation of the genetic data. While some of those data (e.g. those of Rothfels, 1952) can be interpreted entirely on the basis of prezygotic elimination of the male genetic contribution, others (e.g. those of Cavalli-Sforza & Jinks, 1956) could not be, and were not, explained entirely on such a basis but demanded the introduction of the notion of incomplete pairing. Meanwhile the problem of negative interference became an important issue in other microorganisms such as bacteriophage, *Aspergillus*, *Neurospora* and yeast (review, Pritchard, 1960). The most plausible current model of localized negative interference in these organisms postulates that chromosome pairing is discontinuous and random, and that recombination only occurs within those small regions where pairing is effective (Pritchard, 1955; Chase & Doermann, 1958); thus the 'coincidence of recombination in two intervals would occur with greater than random frequency if these intervals were short enough and close enough to be frequently included within one effectively paired segment' (Pritchard, 1960). The mean distance over which such negative interference operates is very small, being of the order of only a few cistrons in both bacteriophage and *Aspergillus* (Pritchard, 1960).

In crosses mediated by phage P1 transduction in *E. coli*, it has been found that when inheritance of the closely linked donor loci, *thr*$^+$ and *leu*$^+$ (ability to synthesize threonine and leucine), is selected, intermediate donor loci such as ara_2 (fermentation of arabinose) are excluded from the *thr*$^+$ *leu*$^+$ recombinants with significant frequency (Lennox, 1955; Gross & Englesberg, 1959) (see Fig. 1). On the other hand, when the same two loci are selected in crosses mediated by conjugation between the same two strains, the intermediate ara_2 locus is virtually never excluded from *thr*$^+$ *leu*$^+$ recombinants (our unpublished data; see also Cavalli, Lederberg & Lederberg, 1953). This anomaly is readily explicable on the above model of negative interference. In transduction, the distance between the selected loci *thr*$^+$ and *leu*$^+$ approaches the maximum length of a transducible fragment (Lennox, 1955), so that the inheritance of these loci must be associated

with recombinational events occurring very close to them with the result that negative interference becomes operative. In conjugation, however, because the segment *thr–leu* is only a small fraction of the total length of the donor chromosome generally transferred to the zygote, there is a high probability that inheritance of this segment as a whole will result from recombination events occurring far from its extremities, i.e. outside those regions where the loci will be affected by negative interference.

On the other hand, some previous studies of conjugal crosses in *E. coli* K-12 (mentioned above) have suggested that negative interference may operate over regions much greater than those of effective pairing as postulated by the current model (Pritchard, 1960). Our attention was directed to this problem by a peculiarity in the inheritance of fimbriation (i.e. possession of non-flagellar appendages called fimbriae) in *E. coli* K-12. Before presenting the data, however, it is necessary to summarize those unique features of chromosome transfer in ♂ Hfr × ♀ F⁻ crosses which are relevant to the present problem.

(1) The cells of any given ♂ Hfr strain conjugate with ♀ F⁻ cells with an efficiency of the order of 100% under optimal conditions and then transfer their single chromosomes in a homogeneous, oriented way, such that one particular chromosomal extremity (*O*) is always the first to penetrate the ♀ cells which thus become zygotes. Thereafter the various ♂ loci enter the zygotes in the order of their arrangement on the chromosome (Wollman *et al.*, 1956). During chromosomal transfer, however, there is a tendency for the chromosome to break, with the result that loci distal to the break do not enter the zygote and cannot appear in recombinants. The probability of any locus being excluded in this way is an exponential function of its distance from the extremity *O* (Wollman & Jacob, 1959).

(2) Under standard conditions, each ♂ Hfr locus begins to enter ♀ F⁻ cells at a very precise time after the commencement of mating. There is good evidence that the speed of chromosomal transfer is constant over the region we have studied (Fuerst, Jacob & Wollman, 1956), so that the distance between loci on the ♂ Hfr chromosome can be precisely mapped as a function of time. Since the time intervals relate to chromosomal transfer, they are independent of subsequent recombinational events, and so can serve as an absolute standard for measuring the frequency of such events in terms of chromosome lengths.

(3) By means of ^{32}P decay experiments the length of chromosome transferred in a given interval of time can be translated into molecular terms (Fuerst, Jacob & Wollman, 1956). It is found that the decay of ^{32}P atoms (incorporated at high concentration) in ♂ Hfr bacteria *prior to mating* prevents them transferring their loci to zygotes at a rate proportional to the distances of these loci from the chromosomal extremity, *O*. Moreover, the relative rates at which transfer of the various loci decline as a function of ^{32}P decay are found to be closely similar to the relative distances, in time units, of these loci from *O*, so that a correlation can be established between genetic distance and ^{32}P decay. From the amount of DNA per nucleus and specific activity of the radio-phosphorus, genetic distance can be the equated with

the number of phosphorus atoms in the chromosomal DNA, provided that two assumptions are made. The first is that the ^{32}P atoms are uniformly distributed among the phospho-diester bonds which constitute the 'backbone' of the DNA double helix. The second is that the probability that a ^{32}P disintegration will prevent further chromosome transfer is the same as that producing death of a bacterium or a phage particle, which is known. Making these assumptions, the data give the result that the whole ♂ Hfr chromosome, whose transfer takes about 100 minutes at uniform speed, contains about 10^7 nucleotide pairs, a figure that agrees well with the amount of DNA per nucleus as estimated by chemical means. Thus approximately 10^5 nucleotide pairs along the double helix of DNA are transferred in one minute at 37° C. (Jacob & Wollman, 1958). This number of nucleotide pairs is of the same order of magnitude as that in the DNA of a phage particle, while the equivalent length of *E. coli* chromosome is also the maximal segment transducible by phage P1.

The genetic data to be presented emerged from crosses between the fimbriated (Fim$^+$) ♂ strain Hfr*H* and the non-fimbriated (Fim$^-$) ♀ strain W945.F$^-$, or derivatives of these strains (see Maccacaro & Hayes, 1961). The relevant loci of ♂ Hfr*H*, the order of their arrangement on the chromosome and the approximate distances between them in terms of times of transfer in broth at 37° are given in Figure 1. The ♀ F$^-$ strain was complementary to the ♂ strain in all these characters.

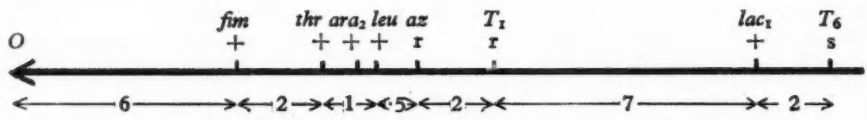


Fig. 1. The relevant segment of chromosome of the ♂ strain Hfr*H*, showing the arrangement of loci and the approximate distances between them (to scale) in terms of times of transfer in broth at 37°. The figures refer to times of transfer in minutes.

O = leading locus; *fim* = fimbriation; *az* = sodium azide resistance (r) /sensitivity(s).

thr = threonine, *leu* = leucine } synthesis

ara$_2$ = arabinose, *lac*$_1$ = lactose } fermentation

T$_1$ = phage T$_1$, *T*$_6$ = phage T$_6$ } resistance (r)/sensitivity (s)

When recombinants inheriting *thr*$^+$ and *leu*$^+$ from the ♂ Hfr parent were selected, 25% were found to be Fim$^-$ and 75% Fim$^+$. This could be interpreted formally in terms of the ratio of the probability of recombination excluding *fim*$^+$ in the short region *fim–thr* (2 minutes) to that of recombination in the much longer region *O–fim* (6 minutes). However, when analysis was made of the distribution of the Fim$^+$ and Fim$^-$ character among the various recombinant classes determined by inheritance of the unselected loci *az*^{r} and *lac*$^+$ on the opposite side of *thr*$^+$ *leu*$^+$, a striking correlation was discovered. The greater the length of ♂ chromosome to the right of *thr*$^+$ *leu*$^+$ that was inherited by recombinants, the higher was the proportion of *fim*$^+$ among them, as Table 1 shows.

Table 1. *Percentage inheritance of the ♂ locus* fim+ *among recombinant classes inheriting various lengths of ♂ Hfr chromosome to the right of the selective loci* thr+leu+. *Mean chromosome lengths inherited are indicated by the uninterrupted lines. Distances are not drawn to scale.*

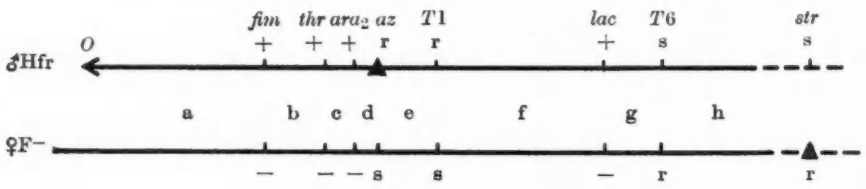
Lengths of ♂ Hfr chromosome inherited by various recombinant classes	*fim*+ inheritance (per cent)	
fim+ *thr*+ *leu*+ *az*r *lac*+	50	Significance in relation to total no. recombinants tested: $\chi^2 = 7{\cdot}55$ P = $<0{\cdot}05$
	68	
	89	

Similar results were obtained in crosses reversed with respect to the *fim* locus, i.e. when the ♂ strain was Fim− and the ♀ strain Fim+, the inheritance of the *fim*− locus was positively correlated ($P < 0{\cdot}05$) with that of increasing lengths of chromosome to the right of *thr*+ *leu*+, showing that the effect was a consequence of the *position* of the locus rather than of the nature of its function. These results seemed to indicate an interaction between recombinational events on either side of the selected locus, such that the closer a crossover occurs to the right of *thr*.*leu*, the greater is the probability of a second crossover between *fim* and *thr*.*leu*, excluding the ♂ *fim* locus. The phenomenon can thus be interpreted as a type of negative interference, but the distances involved are much greater than those over which high, localized negative interference is displayed in *Aspergillus* or bacteriophage. For example, using the data of various authors (Pontecorvo, 1958; Pritchard, 1960; Barricelli & Doermann, 1960), one can estimate that the mean lengths of regions of 'effective pairing' in *Aspergillus* and of 'switch areas' in bacteriophage T4, over which high negative interference occurs, are approximately equivalent to 2×10^4 and 4×10^3 nucleotide pairs of DNA respectively. In comparison, the effect described above extends at least from *fim* to *lac*, a region of the order of 10^6 nucleotide pairs (12 minutes).

Another experiment was therefore performed using a similar cross but embracing a larger number of loci on either side of the selected locus. The genotype of the parental strains with respect to the region of chromosome studied is shown on the map surmounting Table 2, in which the loci are represented at their approximate distances on a time scale. Recombinants were selected for inheritance of the ♂ Hfr locus *az*r (sodium azide resistance) and of the ♀ F− locus *str*r (streptomycin resistance) by plating zygotes, formed in nutrient broth, on nutrient agar + sodium azide + streptomycin. The zygotes were plated before segregation, but after dilution and aeration in nutrient broth + streptomycin for sufficient time to allow full expression of the character *az*r (Hayes, 1957). Since, in this cross, the *str*s locus of the ♂ parent is not transferred to zygotes with significant frequency,

the incorporation of streptomycin in the selective medium merely eliminates ♂ parental growth and has no effect on the constitution of recombinants. The $az^r.str^r$ recombinants were initially classified into two groups determined by recombination (1) in the small regions *c* or *d* (i.e. $az^r.thr^-$), or (2) in the longer regions *a* or *b* (i.e. $az^r.thr^+$). One hundred and thirty-one recombinants from the first group, and one hundred and sixty from the second group were then scored for inheritance of unselected loci from the ♂ parent. An analysis of the results, showing the correlation between the occurrence of recombination in different regions on either side of the selective marker, is given in Table 2. Quadruple recombinants, of which fourteen were among the first group (9%) and seven among the second group (4%), are excluded from the analysis. It should be realized that in systems such as this, where the genetic contribution of one parent is partial, an even number of crossovers is required for the formation of viable recombinants.

Table 2. *Correlation between the occurrence of recombination in different regions on either side of the selective locus* az^r



Analysis of recombinants

A

No. of recombinants with respect to region *a*, *b*, *c* or *d*, and region *e* or $f+g+h$

	e	$f+g+h$	Total	Ratio $e/(e+f+g+h)$
a	10	86	96	0·10
b	12	45	57	0·21
c	17	32	49	0·34
d	30	38	68	0·44
Total	69	201	270	

$\chi^2_3 = 26{\cdot}75$ $P < 0{\cdot}01$

B

No. of recombinants with respect to region *e*, *f* or $g+h$, and region *a* or *b*

	a	*b*	Total	Ratio $a/(a+b)$
e	10	12	22	0·45
f	22	16	38	0·57
$g+h$	64	29	93	0·70
Total	96	57	153	

*$\chi^2_2 = 4{\cdot}73$ $P < 0{\cdot}10$

* The relatively low significance of the data in B is compensated by the concordant results of Table 1, and of many similar (though not identical) crosses.

It will be seen from Table 2A, for example, that when recombination occurs in the interval *d*, close to the left of the selected az^r locus, the ratio of recombination in interval *e* to that in the interval $e+f+g+h$ (0·44) is more than four times higher than that associated with recombination in interval *a* (0·10). A gradient of interaction exists between these two extremes.

In Table 3, the same data are analysed in a different way. In the left-hand column are listed increasing numbers of ♂ Hfr loci located progressively to the right of the selected locus az^r; the other columns give the percentage of recombinants inheriting these ♂ loci, as a function of recombination in intervals *a*, *b* and *c* to the left of the selected locus (see map surmounting Table 2). It is evident that the gradient of inheritance of the loci located to the right of the selected locus is determined by the position of recombinational events to the left, and that this effect extends at least as far as the T_6 locus at a distance of 11 minutes, or approximately one-tenth the length of the whole chromosome, from the selected locus.

Table 3. *The gradient of inheritance by recombinants of loci on the ♂ chromosome distal to the selected locus* az^r, *as a function of proximal recombination regions. The loci and regions are specified in Table 2*

	Percentage recombinants of various classes among recombinants for regions		
Inheritance of ♂ loci	*a*	*b*	*c*+*d*
az^r	100	100	100
$az^r.T1^r$	90	80	59
$az^r.T1^r.lac^+$	67	50	41
$az^rT1^r.lac^+.T6^s$	46	35	26
Total number recombinants scored	(96)	(57)	(117)

This phenomenon, which could not plausibly be interpreted in terms of recombination within small paired regions, suggested a new model of negative interference to account for the extended effect. Like the previous model, it supposes that, before pairing, there is an equal probability that any region of the chromosomes will pair and that this region is small. It further supposes, however, that as soon as pairing has occurred at any region, the probability of pairing at any other region is no longer random but is increased in inverse proportion to its distance from the first region of pairing. It is apparent that this theory, which involves an interaction between *different regions of pairing*, in no way conflicts with the earlier model, but provides a more general framework within which the earlier model can be included. Since the probability of a single recombination event in an effectively paired region has been estimated as about unity in both *Aspergillus* and phage (Pritchard, 1960; Chase & Doermann, 1958), there is no need to postulate that the occurrence of a second, adjacent region of pairing reduces this probability (i.e. that there is a superimposed positive interference) in order to account for the observed results. The alternative possibility that negative interference over large distances might be due to greatly extended regions of effective pairing in a proportion of zygotes, was considered less likely if it is assumed (*a*) that the mechanism of recombination in transduction and conjugation is the same, and (*b*) that, given effective pairing, the probability of a recombinational event per unit dist-

ance remains the same irrespective of the length of the effectively paired region. The probability of such an event excluding the ♂ locus *ara*$^+$ from recombinants selected by inheritance of the two outside loci *thr*$^+$ and *leu*$^+$ (see above) would then remain the same irrespective of whether the region of pairing was small (as in transduction) or large. As has been mentioned, however, the locus *ara*$^+$ is virtually never excluded from such recombinants derived from conjugal matings, so that, if the assumptions are correct, the region of effective pairing is smaller than the region *thr–leu*.

In presenting these results, we have refrained from considering the many factors, such as the possible effect of the ♂ Hfr leading locus (*O*) in promoting pairing, the polarity of chromosomal replication or the occurrence of chromosomal breakage during genetic transfer, which may complicate the recombination process in *E. coli* in ways not yet understood. Whether or not the tentative theory of widespread negative interference which we have suggested is a correct one, we feel that the data themselves confirm the existence of the phenomenon in *E. coli* crosses and that this must be taken into account in recombinational analysis. An answer to the question whether two distinct mechanisms of negative interference operate, or only one, awaits the demonstration of either a continuous or a discontinuous gradient of effect when the interactions of a continuous series of loci, separated by distances ranging from intra-cistronic to those of the order we have examined, are analysed in a single system. Conjugation in *E. coli* is well suited to such a study since it is the only system where recombinational events can be equated with absolute chromosomal lengths.

One of us (G. A. M.) gratefully acknowledges the financial support given him by the Italian Consiglio Nazionale delle Ricerche.

REFERENCES

BARRICELLI, N. A. & DOERMANN, A. H. (1960). An analytical approach to the problems of phage recombination and reproduction: II. High negative interference. *Virology*, **11**, 136–155.

CAVALLI-SFORZA, L. L. & JINKS, J. L. (1956). Studies on the genetic system of *Escherichia coli* K-12. *J. Genet.* **54**, 87–112.

CAVALLI, L. L., LEDERBERG, J. & LEDERBERG, E. M. (1953). An infective factor controlling sex compatibility in *Bacterium coli*. *J. gen. Microbiol.* **8**, 89–103.

CHASE, M. & DOERMANN, A. H. (1958). High negative interference over short segments of the genetic structure of bacteriophage T4. *Genetics*, **43**, 332–353.

FUERST, R., JACOB, F. & WOLLMAN, E. L. (1956). Determination de liaisons génétiques chez *Escherichia coli* à l'aide de radiophosphore. *C. R. Acad. Sci., Paris*, **243**, 2162–2164.

GROSS, J. & ENGLESBERG, E. (1959). Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1bt. *Virology*, **9**, 314–331.

HAYES, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold. Spr. Harb. Symp. quant. Biol.* **18**, 75–93.

HAYES, W. (1957). The kinetics of the mating process in *Escherichia coli*. *J. gen. Microbiol.* **16**, 97–119.

JACOB, F. & WOLLMAN, E. L. (1957). Analyse des groupes de liaison génétique de différentes souches donatrices. *C. R. Acad. Sci., Paris*, **245**, 1840–1843.

JACOB, F. & WOLLMAN, E. L. (1958). Genetic and physical determination of chromosomal segments in *E. coli*. In 'The Biological Replication of Macromolecules', *Symp. Soc. exp. Biol.* **12**, 75–92.

LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*, **1**, 190–206.

MACCACARO, G. A. & HAYES, W. (1961). The genetics of fimbriation in *E. coli*. *Genet. Res.* **2**, 394–405.

PONTECORVO, G. (1958). In *Trends in Genetic Analysis*. Columbia University Press, New York.

PRITCHARD, R. H. (1955). The linear arrangement of a series of alleles in *Aspergillus nidulans*. *Heredity*, **9**, 343–371.

PRITCHARD, R. H. (1960). Localized negative interference and its bearing on models of gene recombination. *Genet. Res.* **1**, 1–24.

ROTHFELS, K. H. (1952). Gene linearity and negative interference in crosses of *Escherichia coli*. *Genetics*, **37**, 297–311.

WOLLMAN, E. L. & JACOB, F. (1959). In *La Sexualité des Bactéries*, p. 158. Masson & Cie, Paris.

WOLLMAN, E. L., JACOB, F. & HAYES, W. (1956). Conjugation and genetic recombination in *Escherichia coli* K-12. *Cold Spr. Harb. Symp. quant. Biol.* **21**, 141–162.

Genet. Res., Camb. (1961), **2**, *pp.* 414–423
With 2 text-figures
Printed in Great Britain

Two linkage groups in *Coprinus lagopus*

By P. R. DAY

John Innes Institute, Bayfordbury, Hertford, Herts.

and G. E. ANDERSON

City of Liverpool College of Technology

(*Received* 29 *April* 1961)

INTRODUCTION

During three years' work on *Coprinus lagopus* a variety of mutants have been isolated, characterized and tested for linkage. The chief objective was to find mutants linked with the two independent factors *A* and *B* controlling the mating system which could be used in studies of their genetic structure. This paper is a summary of our progress.

In recent years *Coprinus* has been the subject of studies of mutation (Fries, 1948; Mittwoch, 1951; Anderson, 1959), cytoplasmic inheritance (Day, 1959), heterokaryosis (Swiezynski & Day, 1960*a* and *b*), the structure of the *A* locus (Day, 1960*a*, *b*) and suppressor action (Lewis, 1961).

MATERIAL

C. lagopus belongs to the family Agaricaceae and is a member of a large and complex genus. There is some confusion between it and the related species *C. cinereus* (*C. fimetarius*). The material used by us conforms closely to Buller's (1924) description of *C. lagopus* and so this name will be used. According to Orton (1957), however, the name *C. cinereus* is correct for such forms isolated from dung.

Seven stocks each derived from single basidiospores have been used. Two, 54 (A_1B_2) and 68 (A_2B_1), were isolated by Prof. D. Lewis in 1952 from a fruit-body found growing on an old chaff heap at Barwick Ford, Herts. The third, 1796 (A_3B_3), was isolated by Lewis in 1955 from a dung-heap at Barlaston, Staffs. The remaining four stocks, H1 (A_5B_5), H2 (A_6B_5), H6 (A_5B_6) and H9 (A_6B_6), were isolated in 1957 from one fruit-body collected on a dung-heap at Bayford, Herts. All crosses between stocks from different fruit-bodies are fertile.

METHODS

Media and culture methods have already been described by Day (1959, 1960*a*). For random spore analysis basidiospores were spread on minimal, or complete medium, containing 0·01% furfural (Emerson, 1954). Incubation was at 28° C.

Mutants were produced by irradiating suspensions of oidia with ultra-violet light to kill 95–99%. The method of filtration enrichment (Catcheside, 1954; Woodward *et al.*, 1954) was used to increase the proportion of auxotrophs.

Mutants with similar requirements were tested for complementation by crossing compatible monokaryotic strains and testing the dikaryon for prototrophy.

RESULTS

The numbers and kinds of mutants isolated are shown in Table 1.

Table 1. *Mutants isolated from H9* (A_6B_6) *wild type*

Number of complementation groups shown in brackets

Requirement or phenotype	No. of mutants
Adenine, hypoxanthine	13 (6)
Adenine (not tested further)	54
Methionine	33 (at least 5)
Choline	1
Choline, dimethyl-aminoethanol	3 (1)
Nicotinic acid	4
Nicotinic acid, anthranilic acid	1
Para-aminobenzoic acid	3 (1)
Arginine	1
Uracil	1
Unknown	2
Cycloheximide resistant	1
Penicillin and oligomycin sensitive	2
Morphological mutants	4 (4)

The following mutants were also isolated from 1796 (A_3B_3) wild type: 12 adenine (5), 3 methionine (3), 1 nicotinic acid.

Linkage tests

Sixty-six independently produced mutants were tested for linkage with the *A* or *B* loci. The bulk of the mutants were induced in H9 (A_6B_6) and these were crossed with H1 (A_5B_5). Six loci are within 20 units of the *A* locus and three are within a similar distance of the *B* locus. Table 2 summarizes the segregations and tests for linkage with *A* or *B* for these loci.

A *linked markers*

The three point crosses to determine the order of the markers in the *A* linkage group are summarized in Table 3.

The four independent mutants requiring *p*-aminobenzoic acid which are linked with the *A* locus are non-complementary in all pairwise combinations. Non-complementary dikaryons between the mutants fruited readily when inoculated to dung supplemented with *p*-aminobenzoic acid. Basidiospores from these

Table 2. *Segregation and recombination of markers linked with* A *and* B

All mutants were induced in stock H9 ($A_6\,B_6$) except 815 (*me*-2) and 838 (*ad*-5) which were induced in stock 1796 ($A_3\,B_3$). Mutants M6 and M2 were isolated by Mr D. Morgan at University College, London.

Mutant	Isolate number	Total progeny	No. of mutants	% recombination with *A*		s.e.
ad-8	1904	401	254†	1·98		0·74
	2242	320	128†	1·27		0·63
	3231	64	35	0·0	(2·43)*	—
	M6	128	75	0·0	(3·22)*	—
me-2	815	877	176†	6·8		2·5
me-5	1905	118	60	34·7		4·4
me-6	2214	96	41	12·6		3·4
paba-1	2519	1184	529†	0·54		0·21
	3445	62	42†	1·2		0·88
	3463	64	38	0·0	(4·60)*	—
	M2	101	54	0·0	(0·45)*	—
arg-1	2246	95	39	6·4		2·5
				with *B*		
ad-5	838	364	236†	7·1		1·6
chol-1	2212	240	135	9·9		2·3
	3245	153	81	15·1		2·9
	3409	64	29	2·2		1·5
chol-2	2222	224	98	11·4		2·7

* 5% upper fiducial limit.
† Differs from a 1:1 ratio at 0·01 probability level.

Table 3. *Estimates of recombination between* A *linked markers based on prototroph frequencies*

The cross *ad*-8 × *arg*-1 was analysed as tetrads, all the progeny of the cross *ad*-8 × *me*-5 were characterized.

Cross a		Cross b	Progeny	Prototrophs	Prototroph *A* mating types a	Prototroph *A* mating types b	% recombination
paba-1 (2519)	×	*ad*-8 (2242)	ca. $1·9 \times 10^5$	2685	721	1774	1·35
ad-8 (2242)	×	*arg*-1	183 (tetrads)	8	0	8	2·18
	×	*me*-2	320	84	2	76	52·5*
	×	*me*-6	256	14	1	13	10·9
	×	*me*-5	96	11	0	11	27·0
arg-1	×	*me*-2	430	37	7	30	17·2
	×	*me*-6	384	43	4	38	22·4
	×	*me*-5	1248	257	13	81	41·18
me-2	×	*me*-5	512	56	4	52	21·8
me-6	×	*me*-5	128	11	6	5	17·2

* Frequency of prototrophs approaches 25% due to segregation of *me*-2 suppressor from *me*-2$^+$ parent.

fruit-bodies gave rise to low frequencies of prototrophs. The prototroph frequencies are shown in Table 4 together with the mating types of randomly selected prototrophs. Approximately 10^6 viable spores were analysed in each cross.

Table 4. *Prototroph frequencies and mating types from crosses between mutants at the* paba-1 *locus*

Cross			Prototrophs per 10^5 viable spores	No. tested	Prototroph mating types		
A_5		A_6			A_5	A_6	$A_?$
2519	×	M2	4·3 ± 0·8	49	4	27	18
	×	3463	9·9 ± 1·4	32	7	12	13
	×	3445	24·4 ± 2·5	32	7	23	2
	×	2519	0·0	—	—	—	—
3445	×	2519	35·9 ± 3·8	22	20	2	0
	×	M2	31·8 ± 6·4	50	48	2	0
	×	3463	29·1 ± 9·8	15	15	0	0
	×	3445	0·0	—	—	—	—
M2	×	2519	0·0	—	—	—	—
	×	3463	0·0	—	—	—	—
	×	3445	18·6 ± 4·6	19	1	15	3
	×	M2	0·0	—	—	—	—

The A mating types of the prototrophs are chiefly those which would be expected if the mutant order were:

—3445——3463——*M*2——2519——*A*—

The same order is indicated by the prototroph frequencies (see Fig. 1). The prototrophs listed as $A_?$ are compatible with both A_5 and A_6 testers. They are the subject of further investigation.

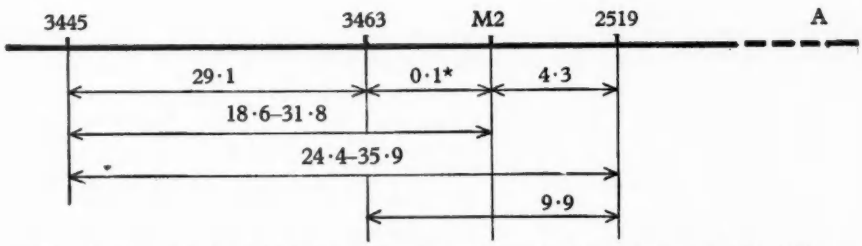


Fig. 1. Map of the *paba*-1 locus showing prototroph frequencies per 10^5 viable basidiospores.
* 5% upper fiducial limit.

The failure of the four *paba* mutants to complement each other and the short length of chromosome which they occupy (*ca.* 0·056 map unit) suggest that they are all mutants at different sites within the same functional unit or cistron (Benzer, 1955) *paba*-1.

All attempts to fruit crosses between the four non-complementary adenine-requiring mutants at the *ad*-8 locus failed.

B *linked markers*

Three point crosses among the *B* linked markers are summarized in Table 5.

Table 5. *Estimates of recombination between* B *linked markers based on prototroph frequencies*

Cross a		Cross b	Progeny	Prototrophs	Prototroph *B* mating types a	Prototroph *B* mating types b	% recombination
chol-1 (2212)	×	*chol*-2 (2222)	256	8	8	0	6·2
,,	,,	,,	52 tetrads	10	10	0	9·6
ad-5	×	*chol*-1 (2212)	454	67	19	48	18·9
				19*	17*	2*	
,,	,,	,,	81 tetrads	2	0	2	1·1
ad-5	×	*chol*-2	$2·7 \times 10^6$	377	190	170	0·028

* Double mutants.

The first two crosses in Table 5 have established the gene orders:

—*B*——*chol*-2——*chol*-1— and —*B*——*ad*-5——*chol*-1—

In the cross *ad*-5 × *chol*-1 the difference between random spores and tetrads is most likely due to the fact that the parent stocks were different in each case. The random spores came from a cross between the original mutants, the tetrads came from a cross between stocks derived from the mutants.

The cross between *ad*-5 and *chol*-2 was made three times and the data from these crosses has been bulked in Table 5. The frequency of prototrophs varied between $2·9 \times 10^{-4}$ and 15×10^{-4} in different fruit-bodies from the same dikaryon fruited at different times and from $1·7 \times 10^{-4}$ to $15·0 \times 10^{-4}$ in different crosses. The *B* mating types of the prototrophs were determined to see if they could be used to place the two markers *ad*-5 and *chol*-2 in a consistent order. Both parental *B* tester stocks were used so that prototrophic dikaryons, common *A* heterokaryons and disomics heterozygous for *B*, which would all be compatible with both testers, could be rejected. Three prototrophs were also crossed to wild type to see if they were disomic or diploid, but homozygous or hemizygous for *B* and thus not detectable by the mating reaction. No auxotrophs were recovered among the progenies of these crosses in tests of at least 100 spores.

The mating types of the prototrophs did not reveal a consistent order for the three markers *B*, *ad*-5 and *chol*-2. We are indebted to our colleague Dr J. R. S. Fincham for suggesting the following explanation. The two mutants were induced in different wild-type stocks; *ad*-5 in 1796 (A_3B_3) and *chol*-2 in H9 (A_5B_5). If the chromosome region carrying the markers *ad*-5 and *chol*-2, but not the centromere, were inverted in one of the mutants, it would follow that, if the two segments paired, a single crossover between the markers would yield duplication-deficiency chromatids which would almost certainly be inviable. A viable prototrophic combination would only result from a two-strand double exchange within

the inversion where one of the crossovers was between the markers. The position of the other crossover, to the right or left, would then determine the *B* mating type of the prototroph. Thus the proportion of one *B* mating type to the other among the prototrophs would depend on the distribution of crossovers within the inversion. This would vary from one cross to another as a result of differences in chiasma distribution consequent on differences in pairing in the mutually inverted segments. The explanation also accounts for the apparent very close linkage between *ad*-5 and *chol*-2.

The three independent mutants at the *chol*-1 locus are non-complementary but dikaryons between different *chol*-1 mutants readily produce fruit-bodies when inoculated to dung. The frequencies of the prototrophs produced and their *B*

Table 6. *Prototroph frequencies and mating types from crosses between mutants at the* chol-1 *locus*

Cross			Prototrophs per 10^5 viable spores	No. tested	Prototroph mating types	
B_5		B_6			B_5	B_6
2212	×	3409	$34{\cdot}5 \pm 5{\cdot}0$	61	41	20
2212	×	3245	$13{\cdot}8 \pm 2{\cdot}1$	56	36	20
3245	×	3409	$41{\cdot}6 \pm 4{\cdot}1$	123	76	47

mating types are shown in Table 6. In each progeny there is an excess of B_5 prototrophs suggesting the order

—*B*——3409——3245——2212—

However the recombination frequencies suggest the order

—*B*——3409——2212——3245—

The most likely reason for this discrepancy is that the recombination frequencies varied in the different crosses because they were subject to differences in genetic background between the crosses.

Although the three *chol*-1 mutants appear to be mutant sites within the same functional unit or cistron their recombination values with the *B* locus vary from 2·2 (3409) to 15·1 (3245) in crosses of the original mutants, induced in H9, with the wild-type stock H1 (Table 2). These crosses and the cross between *chol*-2 (2222) and H1 were repeated using the same stocks as before. At the same time the four mutants were crossed to an A_5B_5 stock which had been produced by backcrossing H1 to H9 six times. We are indebted to Mr D. Morgan for giving us a culture of this backcrossed stock. The results are shown in Table 7. After backcrossing, the recombination frequencies were increased from 2 to 6 times.

Similar effects of backcrossing have been noted by Stadler (1956) and Towe (1958) on the second division segregation frequency of the marker *asco* in *Neurospora crassa*.

Table 7. *The effect of backcrossing on linkage estimation*

	Total progeny	Auxotrophs	% recombination with *B*	S.E.
chol-1				
2212 × H1	145	77	10·0	3·3
× BC*	108	57	21·3	3·9
3245 × H1	87	53	10·1	3·6
× BC	177	92	27·4	3·8
3409 × H1	106	54	5·1	2·2
× BC	183	105	36·5	4·3
chol-2				
2222 × H1	128	60	11·4	3·6
× BC	130	67	20·0	3·6

* BC = H1 (A_5B_5) backcrossed to H9 (A_6B_6) six times.

Mapping the centromeres

Three crosses were analysed as tetrads with the object of mapping the centromeres of the *A* and *B* chromosomes using the method suggested by Whitehouse (1957) for unordered tetrads. This method makes use of the fact that the frequency of tetratype tetrads for two unlinked loci depends on the frequency of second division segregation of the two loci.

If the data include three loci, two of which are linked, then, provided one of the linked loci is close to the centromere, second division segregation frequencies can be calculated. In fact each cross included three linked markers and one unlinked marker. Thus the second division segregation frequencies could be calculated in three ways according to which pair of linked loci were selected. The data on which these calculations are based are given in Table 8.

Table 8. *Numbers of tetratype tetrads for all possible marker pairs obtained in three different crosses*

	Markers a	b	c	d	No. of tetrads	Marker pairs ab	ac	ad	bc	bd	cd
i.	$\frac{A_5}{A_6}$	+	*arg*-1	$\frac{B_6}{B_5}$	180	9	14	72	5	68	66
ii.	$\frac{A_6}{A_2}$	$\frac{B_5}{B_6}$	+	$\frac{\textit{chol}\text{-}1}{+}$	81	11	6	8	7	9	2
iii.	$\frac{A_6}{A_5}$	$\frac{B_5}{B_6}$	*chol*-2	$\frac{\textit{chol}\text{-}1}{+}$	52	13	13	22	6	16	10

If one of the linked markers is close to the centromere, then it is valid to assume that the tetratype frequency for the pair of linked markers is equal to the sum or difference of their second division segregation frequencies. The relative positions of the linked markers and the centromere are determined by trial and error by finding which of three equations gives a real solution.

Among 183 tetrads from cross (i) there were 3 which resulted from two-strand double exchanges between *A* and *arg*-1. These were the only double exchanges and they have been excluded from the calculations. Real solutions to the equations given by Whitehouse were only obtained for the following order of markers on the *A* chromosome:

—*A*——*ad*-8——centromere——*arg*-1—

The tetratype frequencies from cross (ii) did not give a consistent position for the centromere in relation to the *ad*-5 locus. Real solutions to the equations involving the pair of linked markers *B*-*ad*-5 are only obtained if the centromere is assumed to be between them. The same is also true of the equations for the pair *ad*-5–*chol*-1. It was earlier suggested that the *ad*-5 locus may be present in an inversion to explain the low frequency of recombination between it and *chol*-2 and the anomalous recombination with the adjacent *B* marker. Accordingly the data from cross (ii) have been treated assuming that no recombination between *ad*-5 and the centromere took place.

The tetratype frequencies from cross (iii) indicate the marker order

—*B*——centromere——*chol*-2——*chol*-1—

The frequency of recombination between *B* and *chol*-1 in cross (iii) is approximately three times that in cross (ii) (15·4 % compared with 5·5 %), showing that crossing-over is reduced in this region of the *B* chromosome when the *ad*-5 marker is present, which would be expected if it were present in an inversion.

The second division segregation frequencies for *A*, *B* and the linked markers are given in Table 9.

Table 9. *Percentages of second division segregation calculated from tetratype data in Table 8*

Cross	Trio of markers			Percentage second division segregation						
				A	*ad*-8	*arg*-1	*B*	*chol*-2	*chol*-1	*ad*-5
i.	*A*	*ad*-8	*B*	6·6	1·6	—	37·0	—	—	—
	A	*arg*-1	*B*	7·6	—	0·2	36·6	—	—	—
	ad-8	*arg*-1	*B*	—	2·6	0·16	36·6	—	—	—
ii.	*B*	*chol*-1	*A*	6·7	—	—	7·6	—	3·5	—
	B	*ad*-5	*A*	7·4	—	—	8·6	—	—	0*
	ad-5	*chol*-1	*A*	7·4	—	—	—	—	2·5	0*
iii.	*B*	*chol*-1	*A*	23·7	—	—	1·9	—	28·8	—
	B	*chol*-2	*A*	21·0	—	—	5·8	5·8	—	—
	chol-2	*chol*-1	*A*	6·6	—	—	—	20·4	39·6	—

* See text for explanation.

DISCUSSION

The groups of markers linked with the *A* and *B* loci have been mapped. The only inconsistencies in the present data are the relative positions of *ad*-5, *chol*-2 and the centromere and the order of two of the sites within the *chol*-1 locus on the *B* chromosome. Maps of the two linkage groups are shown in Fig. 2.

The difficulty in determining the relative positions of *ad*-5 and *chol*-2 is most likely due to the fact that they were mutants induced in different wild-type stocks. Frost (1961) has analysed the consequences of using different stocks of *Neurospora crassa* and has shown how different genetic backgrounds may have profound effects on recombination frequencies within a given interval. We have now adopted H9 as a standard stock for mutation experiments. When mutants derived from H9 are intercrossed with compatible stocks produced by backcrossing to H9 difficulties due to stock heterogeneity are minimized.

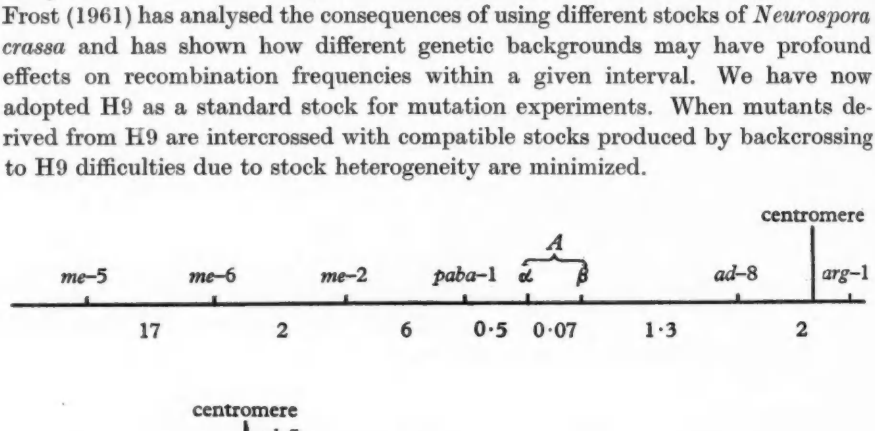


Fig. 2. Maps to show the linear order of the *A* and *B* linked markers.

Some of the variation in recombination between repeats of the same cross (compare Tables 2 and 7) could be attributed to our use of sterilized manure instead of a synthetic fruiting medium. While our stocks of *Coprinus* will fruit on synthetic fruiting medium (Madelin, 1956) they do so more slowly and far less readily than on manure.

The spectrum of mutants obtained in *Coprinus* is broadly similar to those found in other fungi. The two mutants described which were sensitive to penicillin and oligomycin, both of which have no effect on the wild type, are of some interest. They may show a method for studying the mode of action of antibiotics from physiological comparisons between resistant and derived sensitive forms.

The pseudoallelic nature of the *paba*-1 and *chol*-1 mutants is a characteristic feature of most organisms in which the fine structure of individual loci has been studied. All the more interest therefore centres on the basic problem of the fine structure of the *A* and *B* loci for which this work has been a necessary preliminary study.

SUMMARY

Sixty-six independently produced markers have been tested for linkage with the *A* and *B* mating-type factors. Six loci linked with *A* and three linked with *B* were found. Maps of the two linkage groups which include the centromeres were constructed.

Non-identical alleles at two loci, *paba*-1 and *chol*-1, were found and crosses between different mutants were used to map the positions of the mutant sites within each locus.

It is a pleasure to acknowledge the helpful discussions we have had with our colleagues Dr Fincham, Dr Holliday and Mr Gilbert and the valuable technical assistance of Mrs Margaret Lamdin.

REFERENCES

ANDERSON, G. E. (1959). Induced mutants in *Coprinus lagopus*. (Abstr.) *Heredity*, **13**, 411–412.

BENZER, S. (1955). Fine structure of a genetic region in bacteriophage. *Proc. nat. Acad. Sci., Wash.*, **41**, 344–354.

BULLER, A. H. R. (1924). *Researches on Fungi III*. London: Longmans Green & Co.

CATCHESIDE, D. G. (1954). Isolation of nutritional mutants of *Neurospora crassa* by filtration enrichment. *J. gen. Microbiol.* **11**, 34–36.

DAY, P. R. (1959). A cytoplasmically controlled abnormality of the tetrads of *Coprinus lagopus*. *Heredity*, **13**, 81–87.

DAY, P. R. (1960*a*). The structure of the *A* mating type locus in *Coprinus lagopus*. *Genetics*, **45**, 641–650.

DAY, P. R. (1960*b*). Mutations affecting the *A* mating type locu sin *Coprinus lagopus*. (Abstr.) *Heredity*, **15**, 457.

EMERSON, M. R. (1954). Some physiological characteristics of ascospore activation in *Neurospora crassa*. *Plant Physiol.* **29**, 418–428.

FRIES, L. (1948). Mutations induced in *Coprinus fimetarius* (L) by nitrogen mustard. *Nature, Lond.*, **162**, 846.

FROST, L. C. (1961). Heterogeneity in recombination frequencies in *Neurospora crassa*. *Genet. Res.* **2**, 43–62.

LEWIS, D. (1961). Genetical analysis of methionine suppressors in *Coprinus*. *Genet. Res.* **2**, 141–155.

MADELIN, M. F. (1956). Studies on the nutrition of *Coprinus lagopus* Fr., especially as affecting fruiting. *Ann. Bot.* **20**, 307–330.

MITTWOCH, U. (1951). Studies in the genetics of some X-ray induced morphological mutants in *Coprinus lagopus*. *J. Genet.* **50**, 202–205.

ORTON, P. D. (1957). Notes on British agarics 1–5 (observations on the genus Coprinus). *Trans. Brit. mycol. Soc.* **40**, 263–276.

STADLER, D. R. (1956). Heritable factors influencing crossing-over frequency in *Neurospora*. *Microbial Genet. Bull.* **13**, 32–34.

SWIEZYNSKI, K. M. & DAY, P. R. (1960*a*). Heterokaryon formation in *Coprinus lagopus*. *Genet. Res.* **1**, 114–128.

SWIEZYNSKI, K. M. & DAY, P. R. (1960*b*). Migration of nuclei in *Coprinus lagopus*. *Genet. Res.* **1**, 129–139.

TOWE, A. M. (1958). Factors influencing crossing-over in *Neurospora*. *Microbial Genet. Bull.* **16**, 31–32.

WHITEHOUSE, H. L. K. (1957). Mapping chromosome centromeres from tetratype frequencies. *J. Genet.* **55**, 348–360.

WOODWARD, V. W., DE ZEEUW, J. R. & SRB, A. M. (1954). The separation and identification of particular biochemical mutants of *Neurospora* by differential germination of the conidia followed by filtration and selective plating. *Proc. nat. Acad. Sci., Wash.*, **40**, 192–200.

Genet. Res., Camb. (1961), **2**, *pp.* 424–430
With 1 *text figure*
Printed in Great Britain

The ecological genetics of growth in *Drosophila*

V. GENE-ENVIRONMENT INTERACTION AND INBREEDING

By S. S. PRABHU* and F. W. ROBERTSON†
Institute of Animal Genetics, Edinburgh, 9

(*Received* 9 *May* 1961)

1. INTRODUCTION

It is well known that inbreeding in a normally outbred species increases the sensitivity to environmental variation during growth and that this often leads to greater variability in performance. But there is comparatively little information about how arrays of inbred lines derived from the same population differ in their response to controlled differences in the environment. We need to know what particular environmental conditions promote the greatest heterogeneity of response, since this may prove a useful guide to the experimental study of genetic and environmental conditions which influence the stability of development in the outbred population.

The present paper deals with some comparisons between the growth of various inbred lines and the non-inbred parent population in *Drosophila melanogaster* as well as a number of crosses between lines derived from the same population. Controlled differences in environment have been provided by growing larvae, at one or more temperatures, on chemically defined, aseptic media which differ in nutrient composition.

2. MATERIAL AND METHODS

Body size and development time have been recorded on females only and both are expressed on a log scale. Three times the natural logarithm of thorax length is taken as the measure of body size, while development time refers to the duration of the larval period unless otherwise stated. When multiplied by 100, differences on the log scale are roughly equivalent to percentage differences. For ease of reference all differences shown in the tables, relating to both body size and development time, have been multiplied by this factor. The sub-optimal diets have been provided by modifying the more or less complete medium of Sang (1956) by reducing the concentration of such essential nutrients as protein, RNA or fructose. The general experimental procedure and the reasons for choosing these particular diets have been discussed elsewhere (Robertson, 1960).

The lines, inbred for more than fifty generations by brother-sister mating, are derived from the Pacific cage population, and five such lines have been studied.

* Indian Veterinary Research Institute, Izatnagar, U.P., India.
† Agricultural Research Council Unit of Animal Genetics.

Eight to ten females from four to five replicated cultures were generally scored for each genotype or treatment. Unless otherwise stated, heterogeneity between lines in their reaction to different treatments is tested against the error variance of a mean, derived from the pooled within- and between-culture effects; the latter are generally unimportant. The numbers on which the means are based are not quite the same in all cases since occasionally cultures of synthetic medium were infected and had to be discarded. The average number of flies per line per treatment has been used in computing the error variance of a mean.

3. RESULTS

The five inbred lines studied here comprise lines numbered 3, 4, 6, 7, 10. They have been grown at 25° C. and 18° C. on the usual live yeast medium and also two sub-optimal diets: (1) with the protein level reduced from the usual 5% to 2%, and (2) with the concentration of all nutrient halved. In addition, these lines, together with various crosses, have also been grown at 25° C. on media deficient in RNA or lacking fructose. The comparisons between the control population and the five inbreds will be considered first.

Table 1. *Comparisons of body size of Pacific inbred lines and population under different conditions. Deviations from size on live yeast medium*

	25° C.				18° C.	
Genotypes	No Fructose	Low RNA	Diluted	Low protein	Diluted	Low Protein
Line No. 3	−1	0	−17	−29	−25	−33
4	−7	−21	−33	−27	−40	−54
6	−4	−16	−30	−20	−47	−31
7	−7	−32	−27	−49	−47	−75
10	−1	−27	−28	−28	−37	−58
Average of inbreds	−5	−19	−27	−31	−39	−50
Pacific population	−15	−31	−29	−27	−27	−36

Table 1 shows the deviation from the body size on the live yeast medium when flies are grown under alternative conditions. In all sets of comparisons there is significant heterogeneity of differences which may be very great. Thus, on the fructose-free medium, the wild stock suffers the greatest decline—some 15% compared with an average of 5% for the inbreds. Two of the latter, numbers 3 and 10, are quite unaffected by this change in diet. When grown on low-RNA diets, the wild stock is again more affected than the inbred lines, of which No. 3 is unaltered by this treatment which reduces the controls by 30%. On the diluted medium, at 25° C. the inbreds and controls are comparatively alike in their reaction. The inbreds deviate, on the average, by 27—on the × 100 log scale—from their size on the live yeast medium, compared with 29 for the controls.

For the low-protein medium, there is also fair consistency, except for line 7 which suffers a very drastic decline in size compared with the others. At 18° C. the relative performance of the inbreds is clearly inferior to that of the wild stock.

Table 2. *Deviations in body size of Pacific inbred lines from wild population at 25° and 18° C.*

Line	18° C.	25° C.
3	−7*	−18**
4	−9*	−24**
6	3	−21**
7	3	−23**
10	−7*	−24**
Average	−3	−20

* and ** indicate significance at the 0·05 and 0·01 levels of probability.

Evidently the particular chemical composition of the diet determines the magnitude of the heterogeneity of response. Thus low RNA and the diluted medium lead to approximately equal reduction in the body size of the controls but an entirely different array of responses on the part of the inbred lines. On the diluted medium they behave like the controls while on the low-RNA medium they behave quite differently. The general tendency in these comparisons for culture on both diluted and low-protein media to result in greater proportional decline at 18° C. than at 25° C. shows how the effect of a given change in diet is influenced by the temperature during growth. This is apparent even under favourable conditions of culture at 25° C. and 18° C., although the order of effect is reversed. Table 2 lists the deviations from the size of the controls at the two temperatures on the live yeast medium. At the lower temperature the inbred lines are, on the average, only slightly smaller than the controls, whereas at 25° C. they are some 20% smaller. It may be noted that Parsons (1959) has reported gene-environment interaction with respect to survival at different temperatures.

We can now consider the behaviour of the crosses. Lines 3, 4, 7 and 10 and all possible crosses between them have been grown on the live yeast medium and also the fructose-deficient and low-RNA media. In addition, the F_1 of the cross between lines 6 and 10 was grown on all three media while the F_1's between line 6 and lines 3 and 4 were grown on the live yeast alone. So in the latter medium we have altogether nine crosses and seven on each of the other diets. Reciprocal crosses between lines 3 and 4, 3 and 10, and 4 and 10 on the live yeast medium failed to demonstrate any differences in development time, according to the direction of the cross, while for body size only 3×10 showed a difference, just significant at the 0·05 level; the reciprocal crosses have been averaged.

The data are plotted graphically in Figure 1. The main points to note are as follows:

(i) The crosses closely resemble the wild stock in both body size and development time for the three alternative diets. The average deviation from the con-

trols for either body size or development time does not exceed 3% for any of the three diets.

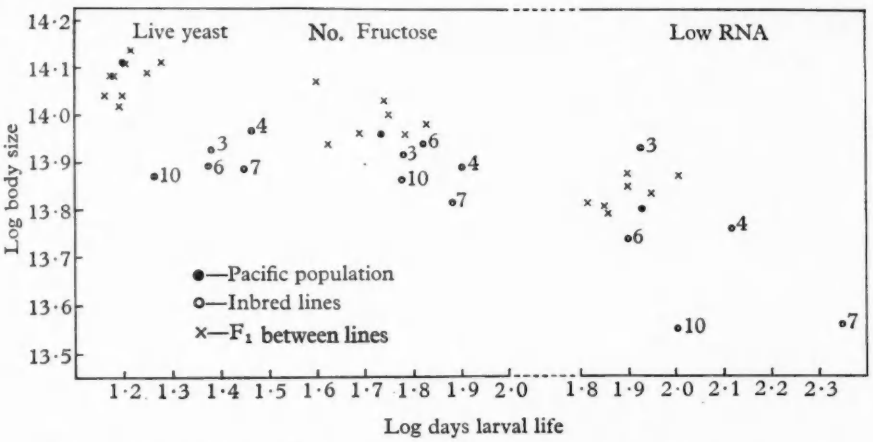


Fig. 1. Mean body size and development time of Pacific inbred lines, crosses and the foundation population, on the favourable live yeast and on two sub-optimal synthetic diets which either lack fructose or are deficient in RNA content.

(ii) It is obvious from Fig. 1 that the crosses, as a class, show much less between-genotype variance than the inbreds. The contrast is particularly striking on the low-RNA diet. A statistical measure of the behaviour of the inbreds and crosses is provided by the estimate of genotype-treatment interaction based on the mean body size of the inbred lines and the six crosses which are represented in all treatments. The results of this analysis are given in Table 3. The gene-environ-

Table 3. *Comparison of gene-environment interaction among inbred lines and crosses*

	Interaction variance		
	d.f.	Mean square	Ratio to error variance
Inbreds	8	51	12·8**
Crosses (*a*)	12	12	3·0*
Crosses (*b*)	10	5	1·2

Crosses (*a*) and (*b*) refer to the analyses for either all crosses or with one excluded.
* and ** indicate significance at the 0·05 and 0·01 levels of probability.

ment interaction is very great among the inbred lines but much lower, although statistically significant, among the crosses. However, inspection of the data shown in Fig. 1 shows that one cross (6 × 10) is unaffected by the change from live yeast to the medium without fructose. This genotype is responsible for a characteristically different response. When the data are recalculated, excluding this cross, the

gene-environment interaction among the crosses is no longer significant, so, unlike the homozygous parents, the heterozygous F_1's, apart from one exception, respond in the same way to the alternative treatments.

(iii) Such contrasts in response on the part of parents and offspring can be looked at from a different angle by estimating the regression of mean F_1 size on the average value of the parents in the different environments. The regressions for the crosses which are represented on all treatments are summarized in Table 4, which also shows the residual variance between means tested against the error variance. For the live yeast medium there is a highly significant regression of progeny mean on mid-parent size of $1{\cdot}60 \pm 0{\cdot}33$, a value of at least unity. Also the residual variance is not statistically significant. Thus, on live yeast, the inbreeding decline which is dissipated by outcrossing is roughly the same for the five lines, and this apparently acts more or less independently of the genetic differences which are responsible for the correlation between F_1 and mid-parent. On the medium lacking fructose the regression, although it works out at 1·0, is statistically quite insignificant; while the heterogeneity of response among the inbreds is so great on the low-RNA diet that all trace of correlation between the size of parents and the size of F_1 vanishes.

Table 4. *Regression of F_1 body size on average of parents*

		Residual variance	
Treatments	Regression	d.f.	Mean square
Live yeast	$1{\cdot}60 \pm 0{\cdot}33$	5	3·7
No fructose	$1{\cdot}00 \pm 0{\cdot}72$	5	18·4**
Low RNA	$0{\cdot}02 \pm 0{\cdot}17$	5	12·4**

The residual variance is tested against the error variance of a mean.
** indicates significance at the 0·01 level of probability.

(iv) A glance at Fig. 1 shows, for any treatment, a distinct negative correlation between size and development time when inbreds are compared with the control wild stock and the crosses. It has been inferred from earlier work (Robertson, 1960) that a decline in body size accompanied by a more or less proportional increase in development time is the best measure of increasingly unfavourable reaction to the diet. The negative relationship in the present data suggests that the effects of inbreeding in reducing body size and lengthening development time are largely due to less successful utilization of nutrients. It is not surprising that the phenotypic effect of inbreeding should be extremely sensitive to changes in the composition of the diet.

DISCUSSION

Although inbred lines are generally more variable in their response to changes in diet than either the crosses between them or the parent population, it does not follow that conditions which lead to smaller adult size or longer period of larval

development necessarily lead to greater proportional changes on the part of the inbreds. This was clearly shown in the comparisons between the Pacific inbred lines and crosses on the media which were deficient in RNA or which lacked fructose. Both media caused much less reduction below the live yeast level on the part of the inbreds than either crosses or wild population. Contrasts of this nature may be related to the inherently faster growth of the non-inbred individuals. This may create a relatively greater requirement for a high concentration of certain essential nutrients. It is worth noting here that lower protein concentration, which leads to roughly similar proportional reduction in body size on RNA-deficient media, was associated with a good deal less heterogeneity of response among the inbreds and the parent population than on the medium with lower RNA. Hence RNA-deficient media may provide especially favourable conditions for the study of gene-environment interaction in relation to diet.

Estimates of the average effects of inbreeding are highly dependent on the diet and temperature during growth. Thus, on the live yeast medium, the Pacific inbreds averaged some 20% smaller than the parent population at 25° C., but only about 3% smaller at 18° C. The parent population and the inbred lines are normally kept at 25° C. and it might be thought that the lines would be better adapted to 25° C. than 18° C. and that the inbreeding decline would be relatively less; clearly this is not so.

There appears to be a high degree of unpredictability in the reaction of inbred lines to changes in diet and temperature. It would be valuable to have comparisons of performance, for a series of different environments, of arrays of inbred lines drawn from a number of different populations. This might afford some evidence of regularity which could never be detected on only a few comparisons.

The crosses show an impressive level of homeostasis in their consistency of response under nutritional conditions which are responsible for wide heterogeneity among the inbred parents. This suggests that the contribution of gene-environment interaction to the phenotypic variance of the foundation population is of a low order. But, on the other hand, Robertson (1960) showed that selection for larger body size on qualitatively different diets quickly leads to striking differences in response to alterations in the composition of the diet. However, the origin of the gene-environment interaction is rather different in the two situations. In the latter case, it originates in selection of genetic differences with greater effect under certain restricted conditions, whereas with the inbred lines we are dealing with numerous unpredictable departures from normal metabolism.

SUMMARY

1. The growth of a number of inbred lines from the Pacific cage population have been compared under different conditions of temperature and nutrition. Body size and duration of the larval period were taken as measures of performance. Sub-optimal diets were provided by growing larvae on chemically defined synthetic media.

2. Gene-environment interaction is widespread and often very great. The phenotypic effects of inbreeding on body size, even on a live yeast medium, may be greatly influenced by temperature. In one set of comparisons, inbred lines averaged 20% smaller at 25° C. but only 3% smaller at 18° C.

3. Sub-optimal diets of different chemical composition, which lead to about the same average decline in body size, may differ greatly in the level of heterogeneity of response among the same set of inbred lines. Thus much greater heterogeneity was found on diets deficient in RNA than on diets with low protein levels. Such information is a useful guide to further study of gene-environment interaction in the outbred population.

4. Diets which lead to a decline in body size of flies of the foundation population do not necessarily cause greater proportional decline on the part of inbred lines. Individual lines have been encountered in which body size is quite unaffected by changes in diet which reduce the size of the outbred flies by 25% or more.

5. A series of crosses between lines from the same foundation population showed a striking level of homeostasis. The average body size and development time of the F_1's was close to that of the population of flies on the favourable and two alternative sub-optimal diets. Also, compared with the parent lines, there was little evidence of gene-environment interaction among the crosses.

REFERENCES

PARSONS, P. A. (1959). Genotypic-environmental interactions for various temperatures in *Drosophila melanogaster*. *Genetics*, **44**, 1325–1333.

ROBERTSON, F. W. (1960). The ecological genetics of growth in *Drosophila*. 1. Body size and developmental time on different diets. *Genet. Res.* **1**, 288–304.

SANG, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *J. exp. Biol.* **33**, 45–72.

Genet. Res., Camb. (1961), **2**, *pp.* 431–438
Printed in Great Britain

Genetical studies on the skeleton of the mouse

XXX. A SEARCH FOR CORRELATIONS BETWEEN SOME MINOR VARIANTS

By GILLIAN M. TRUSLOVE

Medical Research Council Experimental Genetics Research Unit, University College London

(*Received* 11 *May* 1961)

INTRODUCTION

The C57BL strain of the mouse has a large number of minor skeletal variants which are partly under genetical control. This is also true, to a lesser extent, of other inbred strains like CBA and A. In the course of inbreeding, but apparently following the fixation of the initial genetical variance, the C57BL strain has split up into numerous genetically distinct sublines (Deol, Grüneberg, Searle & Truslove, 1957; Carpenter, Grüneberg & Russell, 1957). The differentiation of these sublines is thus due to freshly arising mutations, but the number of these events involving skeletal characters is unexpectedly high. One possible reason for this is that the same mutation may affect more than one skeletal variant and may, therefore, be noted more than once. The 'subline differentiation method' for the estimation of mutation rates, suggested by Deol *et al.* (1957), can thus be validly applied only if it is known to what extent the minor variants used are phenotypically correlated (i.e. have common causes) within the inbred strain, or are genetically correlated (pleiotropism, linkage) in outcrosses.

MATERIALS AND METHODS

A preliminary search for correlations between minor skeletal variants in the C57BL/Gr strain was carried out on sublines V–VII (263 animals). Those correlations which were found to be significant at the 5% level were then reinvestigated in sublines I, II, III and IV (a total of 578 animals). A similar search was carried out using the CBA/Gr strain (489 animals), although there were no sublines available. Lastly, the F_2 generation from a cross between C57BL and CBA (401 animals) was investigated to see if there were any genetical correlations.

Table 1 gives a list of the thirty-one skeletal variants, already described in some of the previous papers of this series, with the percentage incidence in C57BL/Gr sublines V–VII, CBA/Gr and the F_2. Those in italics are characters where a significant correlation was found in one of the strains. With the exception of No. 7 where the relevant bones were not collected in CBA or F_2, the characters marked — did not occur in CBA and failed to segregate out in F_2. Two characters (Nos. 24 and 25) were not included in the C57BL analysis for reasons of convenience. All the variants were treated as all-or-none characters and 2×2 tables were produced by suitable pooling of classes (following the convention used by Deol,

Table 1. *Percentage incidence of skeletal variants in C57BL (sublines V–VII), CBA and F_2*

No.	Variant	C57BL	CBA	F_2
1	Interfrontal	85·6	85·3	66·1
2	Lacrimal-maxilla fusion	15·9	0·8	1·3
3	*Parted frontals*	*7·3*	*75·3*	*44·0*
4	Fused frontals	4·2	—	2·5
5	*Frontal fontanelle*	*7·5*	*35·0*	*3·7*
6	Interparietal-occipital fusion	12·5	—	1·5
7	*Squamosal-parietal fusion*	*7·0*	?	?
8	*Periotic-occipital fusion*	*2·8*	—	—
9	Alae palatinae	72·1	23·9	20·3
10	Foramen ovale single	3·0	63·6	23·2
11	*Foramen ovale open posteriorly*	*3·8*	—	*1·0*
12	Foramen sphenoidale medium	25·7	87·9	23·9
13	*Inframaxillary crest*	*9·4*	*0·9*	*23·8*
14	Processus pterygoideus	17·1	89·7	38·7
15	*Presphenoid, preoptic sutures*	*36·2*	*97·4*	*26·3*
16	*Presphenoid, abnormal metoptic roots*	*1·1*	*72·1*	*29·6*
17	*Foramen hypoglossi single*	*24·8*	*16·4*	*15·6*
18	Accessory mental foramen	26·2	23·0	28·0
19	*Atlas-axis dyssymphysis and/or fusion*	*77·3*	—	*2·5*
20	Other cervical fusions	3·4	—	—
21	Foramina transversaria imperfecta, C III	2·1	—	—
22	*Foramina transversaria imperfecta, C IV*	*34·2*	—	*1·0*
23	*Foramina transversaria imperfecta, C V*	*66·2*	—	*8·5*
24	C VI, tuberculum anterius absent or dystopic	?	1·5	0·5
25	C VI, tuberculum anterius inflexum	?	34·3	1·1
26	*Dystopia of processus spinosus of Th II*	*10·6*	—	—
27	Dyssymphysis of Th I	14·2	—	0·5
28	Dyssymphysis of Th II	0·4	—	1·5
29	Size of processus spinosus of Th II	72·2	57·5	82·3
30	Dyssymphysis of Th X	4·5	—	0·5
31	*L VI sacralized*	*5·9*	*98·9*	*53·5*

Grüneberg, Searle & Truslove, 1960). The first material analysed came from sublines V–VII of C57BL and consisted of 406 fourfold tables. Two-hundred-and-eighty-seven of these were eliminated by inspection as not deviating significantly from proportionality. For the remainder the log likelihood ratio test (the G-test, $G = 2\,\Sigma a(\ln a - \ln m)$, where a is the observed number and m the expected number in a typical cell or class) was calculated from the tables given by Woolf (1957). These values of G were treated as χ^2, and any $\sqrt{G} > 1{\cdot}96$ (corresponding to $P < 0{\cdot}05$) was considered significant. However, if any of the cells in the fourfold tables contained observed numbers less than one, P was calculated by Fisher's exact method. Any correlations found to be significant were reinvestigated using the data from sublines I, II, III and IV. The four $\sqrt{G}$ values thus obtained were added together (taking into account whether they were positively or negatively correlated) and divided by $\sqrt{4} = 2$. Similar analyses were carried out on the data from CBA and F_2, but as there were no sublines present in CBA significant correlations could only be compared with F_2.

ANALYSIS

Table 2 gives the twenty-six $\sqrt{G}$ values judged to be significant in sublines V–VII of C57BL. This is not significantly more than expected on a chance basis (20·3), but nevertheless some genuine correlations may be included in this group. Six significant $\sqrt{G}$ values were repeated by sublines I–IV, but one of these (variants 15 and 31, in the centre column of Table 2) shows a significant positive value in one group of sublines and a significant negative value in the other; for this reason it may be considered spurious. But in the F_2 data there is again a

Table 2. $\sqrt{G}$ *values of twenty-six significant correlations in sublines V–VII compared with those from sublines I–IV. The correlations on the left are negative in both groups of sublines, those on the right are both positive, while those in the centre are positive in one and negative in the other*

Variants	V–VII	I–IV	Variants	V–VII	I–IV	Variants	V–VII	I–IV
	−	−		−	+		+	+
				+	−			
2: 3	2·89	0·72	2:30	2·02	0·68	3: 5	*3·85*	*3·52*
2:18	2·28	0·23	3: 8	2·40	0·08	5: 7	*2·11*	*2·66*
12:21	2·17	0·61	5:13	2·66	0·32	5: 8	3·03	0·35
15:26	2·48	0·49	6:26	2·91	0·87	5:11	2·53	0·79
19:26	*2·18*	*3·21*	8:20	2·63	1·27	7: 8	2·73	0·40
19:31	*2·41*	*2·62*	10:22	2·61	0·38	7:10	3·18	0·16
27:31	2·71	0·24	15:18	2·45	0·45	7:12	2·87	0·40
			15:31	2·05	2·18	8:11	*3·69*	*3·88*
						8:14	1·97	1·56
						14:19	3·54	0·81
						22:30	2·05	1·80

high negative correlation which indicates, perhaps, that this is in fact a genuine case and that the difference between the two groups of sublines is due to differences in the frequencies of the two entities. The other five cases (in italics in Table 2) appear to be genuine correlations, but in none of them is the correlation at all close, the correlation coefficients ($r = \sqrt{(\chi^2/N)} \pm 1/\sqrt{N}$) all being in the range of 0·1–0·3 (Table 3).

Table 3. *Correlation coefficients* (r) *for five pairs of C57BL variants*

No.	Variants	V–VII	I–IV	F_2
3 5	Parted frontals Frontal fontanelle	$+0·31 \pm 0·062$	$+0·17 \pm 0·042$	$+0·12 \pm 0·050$
5 7	Frontal fontanelle Squamosal-parietal fusion	$+0·15 \pm 0·062$	$+0·12 \pm 0·042$	(—)
8 11	Periotic-occipital fusion Foramen ovale open	$+0·30 \pm 0·062$	$+0·23 \pm 0·042$	—
19 26	Atlas-axis fusion Dystopia of p.s. of Th II	$-0·14 \pm 0·062$	$-0·12 \pm 0·042$	—
19 31	Atlas-axis fusion L VI sacralized	$-0·16 \pm 0·062$	$-0·12 \pm 0·042$	$-0·10 \pm 0·050$

For two of the five character pairs in Table 3, independent confirmation comes from the F_2 data. As will be discussed below, the reappearance of a correlation in F_2 may be regarded as supporting evidence for conclusions drawn from the study of the inbred strain, but the converse may not be true. In the remaining three cases, the F_2 data give no information, either because one of the variants involved failed to segregate out in the F_2 generation or, in one case (—), because the relevant bones had not been included in the original collections. The anatomical nature of the correlated character-pairs will be discussed below.

All the cases in Table 3 are, presumably, true correlations. Table 2 may contain additional examples, such as variant pairs 8 & 14 or 22 & 30, and the fact that of the remaining twenty $\sqrt{G}$ values, eleven exceed 2·58 (corresponding to $P = 0{\cdot}01$), perhaps shows that some of these are true correlations as the chance expectation of this number is 4·06. However, as the remaining number of 20 deviations significant at the 0·05 level is practically the same as the chance expectation (20·3), perhaps the matter need not be pursued further.

The CBA strain has a far less variable skeleton than C57BL and only seven 'significant' correlations were found (Table 4). These could only be compared with the F_2 data. One of these seven, preoptic sutures and abnormal metoptic roots of the presphenoid, is paralleled highly significantly in the F_2 data (CBA, $r = +0{\cdot}19 \pm 0{\cdot}045$, and F_2, $r = +0{\cdot}18 \pm 0{\cdot}050$).

Table 4. $\sqrt{G}$ *values of seven significant correlations* ($\sqrt{G} > 1{\cdot}96$) *in the CBA strain together with those in the* F_2 *generation*

Variants	CBA	F_2	Variants	CBA	F_2	Variants	CBA	F_2
	−	−		−	+		+	+
				+	−			
16:29	2·36	1·84	5:15	2·10	1·92	15:16	*4·35*	*3·51*
			10:12	3·05	0·52	15:18	2·06	1·11
			14:17	1·99	0·14			
			15:25	1·99	1·51			

From the F_2 data, twenty-three comparisons were found to be significant at the 0·05 level (Table 5), the chance expectation being 16·25. While the excess of 6·75 is not formally significant it probably includes some genuine correlations. Three F_2 deviations have already been judged to be 'true' correlations on the basis of agreement with the two inbred strains (Tables 3 and 4). For the rest, the greater the deviation, the less likely is it to have arisen by accident alone. The best way to pick out the 'true' correlations, therefore, is to base the selection on the size of $\sqrt{G}$ and proceed in descending order. How far this process should be followed is a matter of choice. Five more $\sqrt{G}$ values exceed 3·2 ($P < 0{\cdot}001$), and may be 'true' correlations. Having thus removed eight from the original total of twenty-three deviations, the fifteen remaining is less than the chance expectation. Moreover, this includes only four values larger than 2·58 ($P < 0{\cdot}01$) which is close to the chance expectation (3·25). It is probably advisable to stop selecting at this point.

Table 5. $\sqrt{G}$ *values of twenty-three significant* F_2 *correlations compared with those in CBA and C57BL (sublines V–VII only)*

Variants	F_2	CBA	C57BL
9:24	−2·11	−1·74	—
12:15	−2·67	−0·61	−1·52
15:31	*−4·00*	−0·77	*−2·05*
3: 5	*+2·35*	+0·51	*+3·85*
3: 9	+2·97	+1·11	+1·00
15:16	*+3·51*	*+4·35*	+1·70
17:19	−2·21	—	−0·99
19:31	*−1·98*	—	*−2·41*
22:31	−2·86	—	−0·84
4: 5	+1·99	—	+0·18
5:22	+2·19	—	+0·36
11:23	*+3·24*	—	+0·62
22:23	*+4·00*	—	+0·99
4:17	−2·21	—	+0·70
17:28	−2·04	—	+1·28
1:23	+2·11	—	−1·93
28:31	+2·46	—	−0·43
5:14	−2·35	+0·78	+1·51
9:13	−2·46	−0·19	+0·70
13:15	*+3·92*	−1·94	+1·46
13:31	−2·88	+0·42	+1·89
14:15	+2·45	−0·32	−0·66
16:17	*+3·77*	−0·39	−0·55

Table 6 gives the actual correlation coefficients (other than those already given in Table 3 and in the text); they are all small, like those found in the inbred strains above.

Table 6. *Correlation coefficients* (r) *for five pairs of* F_2 *variants*

No.	Variants	*r*
15 31	Presphenoid, preoptic sutures L VI sacralized	−0·20 ± 0·050
11 23	Foramen ovale open posteriorly Foramina transversaria imperfecta, C V	+0·20 ± 0·050
22 23	Foramina transversaria imperfecta, C IV Foramina transversaria imperfecta, C V	+0·25 ± 0·050
13 15	Inframaxillary crest Presphenoid, preoptic sutures	+0·18 ± 0·050
16 17	Presphenoid, metoptic roots Foramen hypoglossi single	+0·19 ± 0·050

DISCUSSION

The identification of character-pairs believed to be correlated has been based entirely on statistical evidence. We must now consider to what extent the eleven correlations thus identified are understandable in biological terms. The explana-

tions of some of the correlations appear trivial, of others plausible, of still others adequate, whilst there is also another group which, at present, cannot be explained biologically. These are discussed in more detail below.

(1) In the case of foramina transversaria imperfecta of C IV and C V (Table 6) we are dealing with the same entity manifesting itself in adjacent vertebrae. The information that these two manifestations should be correlated can only be regarded as trivial.

(2) It is plausible that conditions favouring the manifestation of parted frontals will also tend to favour the neighbouring variant of the same suture, the frontal fontanelle (Table 3). Again, it is plausible that the manifestations of preoptic sutures and abnormal metoptic roots of the preshenoid (Table 4) should be correlated, on the grounds of their close proximity, although the exact nature of the common mechanism remains unknown.

(3) The negative correlation between atlas-axis fusion and the dystopia of the processus spinosus of the second thoracic vertebra (Table 3) discovered by Grüneberg (1950) remained inexplicable until it was found by Deol & Truslove (1957) that the former of these two entities is correlated with a low, and the latter with a high, birth weight. The same is true of the other two negative correlations in this series, atlas-axis fusion and sacralization of L VI (Table 3), and preoptic sutures of the presphenoid and sacralization of L VI (Table 6).

(4) While six out of the eleven correlations discussed here are reasonably well understood, the biological meaning of the remaining five is obscure. Nobody familiar with the complexity of biological systems will regard this as an unduly high proportion, nor would it be appropriate to doubt the authenticity of these correlations solely on the grounds that their mechanism is unknown.

Assuming that these eleven correlations are in fact genuine, we have to discuss what they mean in genetical and physiological terms. Correlations between variants within an inbred strain give no information as to whether such correlations are genetical in origin. A positive correlation between two variants A and B may mean that the development of A favours that of B or the reverse, or it may point to a common cause which favours the manifestation of both. A negative correlation between A and B suggests that the circumstances which favour the manifestation of one tend to interfere with that of the other. As shown above, there are six cases which fall into these categories (five in C57BL and one in CBA).

In three of these cases a similar correlation reappears in F_2, indicating that the two variants are either pleiotropic effects of one gene, or that they are the effects of linked genes. While it is impossible to decide which explanation is the correct one from the statistical evidence alone, it may be possible to decide for different reasons. For example f.t.i. in C IV and C V which are correlated in F_2 entered the cross together from C57BL and are the pleiotropic effects of a gene (or combination of genes) with similar effects in adjacent vertebrae. In this particular case the two effects are not significantly correlated in the parent strain, but neither is there a significant correlation between their manifestation on the two sides of the body

(Grüneberg, 1950). In animals genetically capable of showing the character in either vertebra the presence of this phenotype is brought about by local influences which may be described as accidents.

In the remaining three cases, two pairs of the variants which were significantly correlated in C57BL failed to segregate out in F_2. In the last case the relevant bones were not collected in CBA and F_2 and so no further information can be obtained.

The two inbred strains showed 5/406 and 1/136 correlations believed to be genuine, or approximately 1% of all the possible paired comparisons. In the F_2 generation the ratio is a little higher (8/325). The eleven correlations found are all weak, none of them exceeding 0·3. Correlations of less than 0·1 could hardly have been discovered in samples of this size, but some probably exist. The really important fact is that no high correlations between any two entities have been found. This even applies to the F_2 correlation of f.t.i. in C IV and C V, which is an obvious case of pleiotropism. The fact that so many entities should behave independently, or nearly so, is contrary to what one might have expected in an organism or an assembly of closely integrated parts. The reason for this apparent paradox is that these minor skeletal variants are overwhelmingly influenced by chance in their manifestation (Searle, 1954). They constitute the fringe of genetical determination where a slight alteration in the space/time relationships of developing tissues may lead to apparently independent variants.

In any subsequent 'subline differentiation' experiments, the eleven correlations tentatively regarded as genuine, will have to be examined carefully; and differences in sample size taken into account. Conversely correlations found in such subline differentiation experiments may confirm the existence of cases which, on the evidence of the present data alone, have not been established as 'true' correlations.

SUMMARY

A systematic search for correlations between numerous minor skeletal variants in the mouse showed that these are few in number and feeble in extent. This apparent lack of integration is probably due to the fact that these characters are at the extreme limits of genetical determination and so are overwhelmingly influenced by chance in their manifestation.

I should like to thank Professor H. Grüneberg, F.R.S., Dr A. G. Searle and Dr M. S. Deol for allowing me to use their original classifications of some of the skeletons used here, and for advice and encouragement throughout this investigation. I should also like to thank Dr C. A. B. Smith for advice on statistical matters. The support of a grant from the Rockefeller Foundation is gratefully acknowledged.

REFERENCES

Carpenter, J. R., Grüneberg, H. & Russell, E. S. (1957). Genetical differentiation involving morphological characters in an inbred strain of mice. II: American branches of the C57BL and C57BR strains. *J. Morph.* **100**, 377–388.

DEOL, M. S., GRÜNEBERG, H., Searle, A. G. & Truslove, G. M. (1957). Genetical differentiation involving morphological characters in an inbred strain of mice. I: A British branch of the C57BL strain. *J. Morph.* **100**, 345–376.

DEOL, M. S., GRÜNEBERG, H., SEARLE, A. G. & TRUSLOVE, G. M. (1960). How pure are our inbred strains of mice? *Genet. Res., Camb.* **1**, 50–58.

DEOL, M. S. & TRUSLOVE, G. M. (1957). Genetical studies on the skeleton of the mouse. XX: Maternal physiology and variation in the skeleton of C57BL mice. *J. Genet.* **55**, 288–312.

GRÜNEBERG, H. (1950). Genetical studies on the skeleton of the mouse. I: Minor variations of the vertebral column. *J. Genet.* **50**, 112–173.

SEARLE, A. G. (1954). Genetical studies on the skeleton of the mouse. IX: Causes of skeletal variation within pure lines. *J. Genet.* **52**, 68–102.

WOOLF, B. (1957). The log likelihood ratio test (the *G*-test). *Ann. hum. Genet., Lond.* **21**, 397–409.

Genet. Res., Camb. (1961), **2**, *pp.* 439–448
Printed in Great Britain

Linear heritability estimates

BY HANS ABPLANALP

Poultry Husbandry Department,
University of California, Davis

(*Received* 16 *May* 1961)

INTRODUCTION

The use of regression and variance analysis in population genetics is generally accepted as a means of predicting genetic gains from selection. When only one trait is considered these predictions are usually formulated in terms of heritability, h^2, the linear regression of genotype on measured phenotype, in closed populations, and the selection differential, ΔP. Thus in the simplest case of mass selection in large randomly reproducing populations the anticipated response to selection is

$$\Delta G = \Delta P . h^2$$

Selection usually is by truncation, that is the best of a given generation are retained as parents.

The use of linear regression of genotype on phenotype for prediction of change from selection is only justified when such a linear relationship can reasonably be expected on genetic grounds. This implies, in most instances, the assumption that genes act additively, that gene effects are small, that parental effects on offspring are due to such genes only, and that environmental effects are random for individual offspring. These assumptions will apply throughout this paper.

Heritability estimates based on intraclass correlations have by and large proved useful for predicting gains from selection (Clayton *et al.*, 1957). In some respects, however, the conventional concept of heritability has been found wanting.

In actual selection experiments it is often found that response for a given absolute selection differential is greater in one direction than in the other. Thus for growth rate in mice it has been found that selection response in the direction of small body size is greater than in the direction of large size (Falconer, 1960*a*). Clearly a heritability estimate based on the usual variance analysis is incapable of describing such situations, while conventional selection experiments used under those circumstances involve data from at least two generations.

In this paper a method of estimating heritability and genetic correlation is presented which permits evaluation of genetic gains from selection in either direction on the basis of data from a single generation.

HERITABILITY ESTIMATES

Consider a breeding experiment typical for poultry in which sires (s) are each mated to dams (d), each of which gives rise to offspring (g). The latter are grown

together and measured for a given production trait, x. The mathematical model appropriate for analysis of this experiment is:

$$x_{ijk} = m + s_i + d_{ij} + f_{ijk}$$

where x_{ijk} is the measured performance of the kth offspring from ith sire and jth dam mated to that sire;

m the overall mean common to all observations;

s_i the effect of the ith sire on its progeny, presumed to be mostly genetic;

d_{ij} the effect of the jth dam on its offspring, due primarily to genetic causes, but also to non-genetic maternal effects;

f_{ijk} the effect of environment and unpredictable genetic factors affecting the kth offspring from dam j and sire i.

On this model the least-squares estimates for sire, dam, and offspring effects respectively are:

$$\begin{aligned} \hat{s}_i &= x_{i..} - x_{...} = \text{estimated sire effect,} \\ \hat{d}_{ij} &= x_{ij.} - x_{i..} = \text{estimate of dam effects,} \\ \hat{f}_{ijk} &= x_{ijk} - x_{ij.} = \text{estimate of individual offspring effects.} \end{aligned}$$

Small x's denote averages taken over all subscripts represented by a dot.

Assuming that all effects with the exception of m are random samples from given distributions (Eisenhart Model 2), we have the following variances of these estimates:

$$\left.\begin{aligned} \text{Var}\,(\hat{s}) &= \sigma_f^2/dn + \sigma_d^2/d + \sigma_s^2 \\ \text{Var}\,(\hat{d}) &= \sigma_f^2/n + \sigma_d^2 \\ \text{Var}\,(\hat{f}) &= \sigma_f^2 \end{aligned}\right\} \qquad (1)$$

where σ_s^2, σ_d^2 and σ_f^2 are variance components due to sire, dam and individual effects respectively.

Using these relations and estimates of variance components the geneticist then proceeds to predict genetic changes from selection in the following generation by means of heritability estimates (in this case intraclass correlations) and known selection differentials.

It should be noted that linear prediction equations of the type discussed do not rely directly on estimates of variance components, but take the general form:

$$\text{Expected displacement} = \left\{\begin{matrix}\text{Selection} \\ \text{differential}\end{matrix}\right\} \left\{\begin{matrix}\text{Regression of response} \\ \text{criterion on selection} \\ \text{criterion.}\end{matrix}\right\}$$

In the context of the paper the term displacement is used to denote genetic or phenotypic changes from selection by truncation in either direction.

While variance components are a convenient means of estimating the regressions involved, it will be shown that they are not necessary for that purpose. Regressions

can be estimated, in principle, from the expected displacements in component parts of the selection criterion (x) as caused by a constant selection differential (ΔP) of the latter. To this end we define the following displacements and partial regressions, within a generation of pedigreed offspring, and in terms of mass selection on x with selection differential, ΔP:

$$\left.\begin{aligned} \Delta S &= \Delta P(\sigma_s^2/\sigma_x^2) = \text{Displacement component due to sire effects,} \\ \Delta D &= \Delta P(\sigma_d^2/\sigma_x^2) = \text{Displacement component due to dam effects,} \\ \Delta F &= \Delta P(\sigma_f^2/\sigma_x^2) = \text{Displacement component due to individual hen effects,} \end{aligned}\right\} \quad (2)$$

where σ_x^2 is the phenotypic variance of x.

It follows easily that

$$\frac{2(\Delta S+\Delta D)}{\Delta S+\Delta D+\Delta F} = \frac{2(\sigma_s^2+\sigma_d^2)}{\sigma_s^2+\sigma_d^2+\sigma_f^2} = h^2,$$

the usual heritability estimate based on sire and dam components of variance.

Unfortunately the expected displacements of sire, dam and hen effects are not directly observable. They can be derived, however, from displacements in respective least-squares estimates, $\hat{s}_i$, $\hat{d}_{ij}$ and $\hat{f}_{ijk}$. Thus we have:

$$\left.\begin{aligned} \Delta P_S &= \Delta P\frac{\sigma_s^2+\sigma_d^2/d+\sigma_f^2/nd}{\sigma_x^2} = \text{Expected displacement in estimates of sire effects } (\hat{s}_i); \\ \Delta P_D &= \Delta P\frac{(\sigma_d^2+\sigma_f^2/n)}{\sigma_x^2} = \text{Expected displacement in estimates of dam effect } (\hat{d}_{ij}); \end{aligned}\right\} \quad (3)$$

and

$$\left.\Delta P_F = \frac{\Delta P(\sigma_f^2)}{\sigma_x^2} = \Delta F = \text{Expected displacement component due to individual hen effects } (\hat{f}_{ijk}).\right\}$$

The regressions in the above equations follow directly from the variances of the estimates of sire, dam and hen effects respectively as shown in (1). Furthermore, from (2) and (3) we obtain

$$\Delta S = \Delta P_S - \Delta P_D/d \qquad \text{and} \qquad \Delta D = \Delta P_D - \Delta P_F/n.$$

Heritability can thus be determined as a simple function of ΔP_S, ΔP_D and ΔP_F, which can be obtained from the data as follows:

$$\left.\begin{aligned} \Delta P_S &= \frac{\sum_{m=1}^{q}(\hat{s}_{mi.})}{q} = \frac{\sum_{m=1}^{q}(s_{mi..}-x_{m...})}{q} = \text{Average of estimates of sire effects for the } q \text{ selected individuals.} \\ \Delta P_D &= \frac{\sum_{m=1}^{q}(\hat{d}_{ij})}{q} = \frac{\sum_{m=1}^{q}(x_{mij.}-x_{mi..})}{q} = \text{Average of estimates of dam effects for the } q \text{ selected individuals.} \\ \Delta P_F &= \frac{\sum_{m=1}^{q}(\hat{f}_{mijk})}{q} = \frac{\sum_{m=1}^{q}(x_{mijk}-x_{mij.})}{q} = \text{Average of estimates of individual hen effects for the selected individuals.} \end{aligned}\right\} \quad (4)$$

Note the total selection differential from mass selection

$$\Delta P = \frac{\sum_{m=1}^{q} (x_{mijk} - x_{m\ldots})}{q} = \Delta P_S + \Delta P_D + \Delta P_F.$$

As a logical extension of the above development we can define

$\Delta G = 2(\Delta D + \Delta S)$, or $\Delta G = 4\Delta S$, or $\Delta G = 4\Delta D$, as the component of total genetic displacement;

$\Delta M = \Delta D - \Delta S$ as the component of displacement from maternal effects when the latter can reasonably be assumed; and

$\Delta E = \Delta F - \Delta S - \Delta D$ as the component of displacement due to random environmental effects acting on individuals.

This is in accordance with similar definitions based on variance components. It should be noted that the above operations on displacement estimates ΔP_S, ΔP_D and ΔP_F which lead to heritability and other regressions are identical with the calculations applied to corresponding variances, in the usual estimation procedures using variance components.

The present approach to estimation of heritability can easily be extended to cases where selection is on family means or some other known functions of observed performance data. Because the present estimates are simple linear functions of observations it appears appropriate to call them linear heritability estimates. In order for such a linear heritability estimate to be equivalent to one obtained by variance component techniques it would be necessary that the regression of genotype on phenotype be linear. In practice this assumption may not be met. In that case the estimates proposed here are likely to be more realistic than conventional ones in the sense that they are defined directly on changes from selection. They may be visualized as an attempt at fitting a straight line through the origin on one hand and the expected genotype of selected parents on the other.

The present approach to the problem of selection response also has the advantage of relatively few assumptions. Furthermore, we may use it to investigate the symmetry of selection response with data from only one generation of pedigreed individuals.

ESTIMATES OF GENETIC CORRELATION BETWEEN TRAITS

The same method, as applied to one characteristic, lends itself in principle to an investigation of correlated responses in two or more traits. Suppose we are concerned with selection on trait x and wish to predict the consequences of such action for trait y. This can be done by applying mass selection, by truncation, to x at an intensity and direction for which correlation estimates are of interest. For the selected individuals one can then determine average displacement components of y, the correlated trait.

Accordingly we denote the total phenotypic displacement in y due to selection on x as $\Delta P_{y \cdot x}$, and partition it into average displacements from sire effects ($\Delta P_{Sy \cdot x}$),

dam effects ($\Delta P_{Dy.x}$) and individual effects ($\Delta P_{Fy.x}$) respectively, based on least-squares estimates of sire, dam and individual effects, in y, for the breeders selected. As in the case of direct response for a single trait we have for the correlated displacement:

$$\Delta P_{y.x} = \Delta P_{Sy.x} + \Delta P_{Dy.x} + \Delta P_{Fy.x},$$

and correlated displacement components derived by operations identical to those used for direct response in x thus are:

$$\Delta F_{y.x} = \Delta P_{Fy.x},$$

$$\Delta D_{y.x} = \Delta P_{Dy.x} - \frac{\Delta P_{Fy.x}}{n},$$

and

$$\Delta S_{y.x} = \Delta P_{Sy.x} - \frac{\Delta P_{Dy.x}}{d}.$$

Also the total correlated displacement in the genotype of y may be defined as

$$\Delta G_{y.x} = 2(\Delta S_{y.x} + \Delta D_{y.x})$$

and the correlated response due to non-genetic factors

$$\Delta E_{y.x} = \Delta F_{y.x} - \Delta D_{y.x} - \Delta S_{y.x}.$$

By this procedure we thus obtain directly the expected genetic change in y as induced by selection on x or vice versa. These estimates may be all that is required in actual experimentation. In order to compare results from different populations, however, one may proceed to calculate genetic correlations. In case of response in y due to selection on x we define the genetic correlation as

$$r_G = \frac{\operatorname{cov} G_{y.x}}{\sigma_{Gx}\sigma_{Gy}}.$$

We now express genetic variances and covariances as functions of selection differentials and genetic displacements. For normally distributed x we have for the selection differential

$$\Delta P_x = \bar{\imath}.\sigma_x, \tag{5}$$

where $\bar{\imath}$ is the selection differential for truncation in the standard normal distribution.

The genetic displacement in x due to selection on x,

$$\Delta G_x = \frac{\sigma^2_{Gx}}{\sigma^2_x}\Delta P_x,$$

where σ^2_{Gx} is the genetic variance in x.

Substituting σ^2_x in (5) we then have

$$\sigma^2_{Gx} = \frac{\Delta G_x \Delta P_x}{\bar{\imath}^2},$$

and similarly

$$\sigma^2_{Gy} = \frac{\Delta G_y \Delta P_y}{\bar{\imath}^2}.$$

For the genetic displacement in y due to selection on x we have

$$\Delta G_{y.x} = \frac{\operatorname{cov} G_{y.x}}{\sigma_x^2} \Delta P_x$$

hence

$$\operatorname{cov} G_{y.x} = \frac{\Delta G_{y.x} \Delta P_x}{i^2}$$

and

$$r_{Gy.x} = \frac{\Delta G_{y.x}}{\Delta P_y} \Big/ \sqrt{\left[\frac{\Delta G_x}{\Delta P_x} \frac{\Delta G_y}{\Delta P_y}\right]}$$

Alternately we can define genetic correlation on the basis of genetic change in x due to selection on y as

$$r_{Gx.y} = \frac{\Delta G_{x.y}}{\Delta P_x} \Big/ \sqrt{\left[\frac{\Delta G_x}{\Delta P_x} \frac{\Delta G_y}{\Delta P_y}\right]}$$

From what has been said earlier it should be clear that this definition further depends on the direction in which the primary variable is selected.

The present method of estimating genetic correlations deals with a problem for which adequate statistical tools have not been provided by genetic theory so far. According to the conventional statistical formulation of correlated response the genetic correlation between characters is symmetrical, that is, independent of the fact which of the two traits is subjected to selection. With the present method we may subject this assumption to an empirical test, by comparing a given correlation estimate, when obtained from genetic changes in y as induced by selection on x, with comparable changes in x brought about by selection on y. Again limited empirical information from selection experiments suggests that such alternate correlations may be different from each other (Falconer, 1960).

The present method of estimating selection response with data from one generation can readily be extended to other experimental designs than the one considered here, provided such selection has operational meaning. In general this would imply the assumption of selection on unknown random effects for which the experiment is capable of providing least-squares estimates. The present estimation technique can also be adapted to situations where dominance is assumed, in a manner analogous to corresponding analyses of variance.

It should be noted, however, that the present method of partitioning the selection differential (ΔP_x) according to assumed genetic effects breaks down, when the total selection differential becomes small. Empirical results obtained so far suggest that selection differentials of about one standard deviation (σ_x) are giving results comparable to those obtained from conventional variance components techniques. Clearly the method of estimation proposed here is only of interest, when the assumption of linear regression of genotype on phenotype is violated. Under such circumstances non-linearity of regressions would bias predictions, primarily with large selection differentials. But, for relatively weak selection, the conventional intraclass correlations and offspring parent regressions might be preferable to the ones proposed here.*

* It has been pointed out by a referee that the optimum selection intensity of estimating linear heritability for a given set of data must be close to 0·27, the known optimum selection intensity for an ordinary selection programme. (See Robertson, 1957.)

Nothing has been said so far about the statistical properties of the proposed estimates. A derivation of exact distribution functions for either heritability or genetic correlation estimates has not been possible, so far. However, approximate sampling distributions for population structures of particular interest might be obtained on high-speed computers. So far, we have derived heritability estimates from a number of small populations. The results indicate relatively good agreement for heritability based on intraclass correlations and the present estimates, as illustrated by the numerical example given below. Genetic correlations based on populations of only few parents, however, have shown large sampling variance. Thus, in case of relatively small populations there remains the question as to the usefulness of the proposed estimation technique for correlation analysis. However, in case of poultry populations where data are often available from many and large populations it may be desirable to gain further insight into the genetic situation at the expense of some precision of estimates. In such situations the present method of estimation may be useful. Also it requires much less computational work than comparable variance analyses and can thus serve for a quick appraisal of the genetic situation.

A NUMERICAL EXAMPLE

From a large population of pedigreed SCWL hens a random sample of records was drawn so that twenty mated dams were each represented by five offspring. The dams themselves had been mated to individual sires in five groups of four, respectively. We shall now consider part-time egg production records to 40 weeks of age (x) and 40 week egg weight (y) for the 100 hens sampled.

Table 1 shows egg production and egg-weight records of the twenty hens with highest egg number (x), ranked according to the latter. Also given are least-squares estimates of sire effects ($\hat{s}$), dam effects ($\hat{d}$) and individual hen effects ($\hat{f}$) for both traits, based on the entire sample of 100 hens. Average phenotypic displacements in sire effects, dam effects and individual effects are shown in the bottom row of the table; thus, for example,

$$\Delta P_{Sx} = 2{\cdot}13 \text{ eggs, etc.},$$

and correlated displacements in y due to selection on x are

$$\Delta P_{Sy \cdot x} = -0{\cdot}13 \text{ grams egg weight, etc.}$$

Results comparable to those shown in Table 1 were also computed (but are not shown here) for the twenty hens with highest egg weights from which average phenotypic displacements for egg weight (y) and correlated phenotypic displacements in egg number (x) were obtained.

We may now calculate displacement components due to sires, dams and individuals respectively for both direct response to selection and correlated responses. For egg number we thus have the sire component

$$\Delta S_x = \Delta P_{Sx} - \Delta P_{Dx}/d = 2{\cdot}13 - 4{\cdot}91/4 = 0{\cdot}90 = \Delta G/4,$$

Table 1. *Selected breeding animals and corresponding phenotypic displacement*

	Egg number to 40 weeks			Egg weight (g.) at 40 weeks		
Selection criterion x_{ijk}	Sire effects $x_{i..}-x_{...}$	Dam effects $x_{ij.}-x_{i..}$	Individual hen effects $x_{ijk}-x_{ij.}$	Sire effects $y_{i..}-y_{...}$	Dam effects $y_{ij.}-y_{i..}$	Individual hen effects $y_{ijk}-y_{ij.}$
39·06	8·51	10·20	20·35	−0·74	−0·53	1·44
33·81	−1·57	4·59	30·79	0·82	0·34	2·98
29·06	8·51	7·65	12·90	−0·74	−1·84	0·75
29·01	2·23	2·13	28·91	−1·13	−2·29	2·83
26·81	−4·60	2·73	28·68	−0·01	−0·29	0·46
25·04	2·23	4·28	18·53	−1·13	1·54	−1·85
24·31	−4·55	10·85	18·01	1·07	−0·01	2·50
23·06	−1·57	6·48	18·15	0·82	−1·23	−2·42
21·31	−1·57	4·39	18·49	0·82	0·74	−1·00
20·31	8·51	10·20	1·60	−0·74	−0·53	−1·17
19·81	8·51	10·20	1·10	−0·74	−0·53	3·45
19·06	2·23	4·62	12·21	−1·13	−0·17	−1·53
19·06	8·51	−6·56	17·11	−0·74	−0·69	−2·40
18·81	8·51	−11·31	21·61	−0·74	3·04	−0·12
18·81	−4·55	2·15	21·21	1·07	−0·05	2·16
18·31	8·51	7·65	2·15	−0·74	−1·84	1·14
17·31	−4·60	2·73	19·18	−0·01	−0·29	0·84
17·06	−4·55	10·85	10·76	1·07	−0·01	1·11
16·31	8·51	7·65	0·15	−0·74	−1·84	−2·86
16·31	−4·55	10·85	10·01	1·07	−0·01	−6·50
Average						
22·63	2·13	4·91	15·59	−0·13	−0·32	−0·31
(ΔP_x)	(ΔP_{Sx})	(ΔP_{Dx})	(ΔP_{Fx})	$(\Delta P_{Sy.x})$	$(\Delta P_{Dy.x})$	$(\Delta P_{Fy.x})$

Table 2. *Components of displacement, heritabilities and genetic correlations from mass selection on egg number and egg weight in either direction*

Selection method	Displacement components in egg number due to			Displacement components in egg weight due to			Heritability estimates	Estimates of genetic correlations
	sire	dam	individual	sire	dam	individual		
High egg number	0·90* (ΔS_x)	1·79 (ΔD_x)	15·60 (ΔF_x)	−0·05 ($\Delta S_{y.x}$)	−0·26 ($\Delta D_{y.x}$)	−0·31 ($\Delta F_{y.x}$)	0·29 (h^2_x)	−0·63 ($r_{Gy.x}$)
Low egg number	−0·29	1·23	−19·44	0·01	0·23	−0·07	−0·10	−1·07
High egg weight	−1·26 ($\Delta S_{x.y}$)	−0·78 ($\Delta D_{x.y}$)	−5·48 ($\Delta F_{x.y}$)	0·32 (ΔS_y)	0·26 (ΔD_y)	2·26 (ΔF_y)	0·41 (h^2_y)	−0·68 ($r_{Gx.y}$)
Low egg weight	1·04	1·25	−3·21	−0·03	−0·26	−2·90	0·18	−1·77

* Corresponding symbols are given in brackets below numerical values; the same symbols are used for downward selection as in upward selection.

the dam component

$$\Delta D_x = \Delta P_{Dx} - \Delta P_{Fx}/n = 4{\cdot}91 - 15{\cdot}60/5 = 1{\cdot}79 = \Delta G/4,$$

and the individual component

$$\Delta F_x = \Delta P_{Fx} = 15{\cdot}60 = \Delta G/2 + \Delta E.$$

Finally we obtain the total genetic displacement $\Delta G_x = 2(\Delta S_x + \Delta D_x) = 5{\cdot}38$; and the displacement due to environmental effects $\Delta E_x = 12{\cdot}91$; from which we can calculate a heritability

$$h_x^2 = \frac{2(\Delta S_x + \Delta D_x)}{\Delta G_x + \Delta E_x} = \frac{5{\cdot}38}{18{\cdot}29} = 0{\cdot}29.$$

A summary of results obtained for the present example is given in Table 2. Also included in the summary are heritabilities and genetic correlations for selection of either x or y in the downward direction at a selection intensity of one in five.

A comparison of the heritabilities and genetic correlation estimates in Table 2 with corresponding estimates derived from variances shows them in good agreement, in the sense that the variance estimates lie between the two displacement estimates derived for up and down selections respectively. Thus heritability based on variance components is 0·25 for egg number, and 0·31 for egg size, and the genetic correlation is −0·97.

SUMMARY

A method for obtaining linear estimates of heritability and genetic correlation is given. It is based, essentially, on selecting, from a pedigreed population, prospective parents for which estimates of average genotype and phenotype values are obtained; a regression of genotype on phenotype value is then determined from a straight line fitted through points representing the population mean genotype and phenotype on one hand and the mean genotype and phenotype of selected parents on the other.

The method permits an evaluation of asymmetry in response for a trait selected in both directions, as well as asymmetry in correlated response to selection of two different traits, with data from a single pedigreed population.

REFERENCES

CLAYTON, G. A., MORRIS, J. A. & ROBERTSON, A. (1957). An experimental check on quantitative genetical theory. *J. Genet.* **55**, 131–151.

FALCONER, D. S. (1960*a*). Selection of mice for growth on high and low planes of nutrition. *Genet. Res.* **1**, 91–113.

FALCONER, D. S. (1960*b*). The genetics of litter size in mice. *J. cell. comp. Physiol.* **56** (suppl. 1), 153–167.

ROBERTSON, A. (1957). Optimum group size in progeny testing and family selection. *Biometrics*, **13**, 442–450.

Genet. Res., Camb. (1961), **2**, *pp.* 449–455
Printed in Great Britain

Erythrocytic antigenic differences between individuals of the deer mouse, *Peromyscus maniculatus**

By DAVID I. RASMUSSEN
Mammalian Genetics Center, Department of Zoology, University of Michigan, Ann Arbor, Michigan

(*Received* 8 *June* 1961)

All previously published detailed reports of erythrocytic antigenic variation within the genus *Peromyscus* have employed for absorption pooled cells from several individuals thereby hindering the detection of antigenic individuality. Gorer (1936), when investigating antigenic individuality in the house mouse, *Mus musculus*, avoided the problems of small blood volume and individual variation by using pooled cells from inbred (sibship mating) lines. Species specific erythrocytic antigenic differences between deer mice of the species *Peromyscus maniculatus* and *P. leucopus* were demonstrated by Moody (1941) using immune rabbit sera. Cotterman (1944) found that normal human group-B sera absorbed with pooled erythrocytes from individuals of various species within the genus *Peromyscus* possess agglutinins showing quantitatively different reactions against erythrocytes of various *Peromyscus* species. Moody (1948) demonstrated differential cellular antigenic components between populations of the species *Peromyscus maniculatus*. These cellular antigens were demonstrated with the use of immune rabbit sera produced by inoculation of pooled erythrocytes from mice of a given stock. The immune sera were absorbed with pooled blood obtained from several mice of various stocks.

The following study concerns individual erythrocytic antigenic variation in the deer mouse, *Peromyscus maniculatus*, demonstrated by hemagglutination tests using unabsorbed isoimmune mouse sera and immune rabbit sera absorbed with erythrocytes from individual deer mice. Two antigenic types have been detected which appear as unitary genetic characteristics inherited as a two-allele, one-locus system. These two antigens are designated antigen A and antigen B and are not to be considered antigenically related to any other AB antigen system including the familiar A-B antigen system of humans. The alleles at the locus responsible for these antigenic phenotypes are designated as Pm^A and Pm^B.

MATERIALS

Deer mice used for injection and absorption were obtained from laboratory stocks maintained and kindly provided by Dr Elizabeth Barto, Department of Zoology, the University of Michigan. The individuals, of the subspecies *Pero-*

* The investigations reported are a portion of the thesis requirement to be submitted for the degree of Doctor of Philosophy in the Rackham Graduate School of the University of Michigan.

Aided in part by a Rackham Predoctoral Fellowship, University of Michigan, and by a Graduate Genetics Traineeship (USPHS 2G–71 (C2S1)).

myscus maniculatus gracilis (LeConte), represent the fifteenth to twenty-fifth generation descendants of feral animals trapped in Alger County, Michigan, in 1940 by W. Frank Blair and in 1947 by Van T. Harris. The stock has not been purposely inbred, although inbreeding has resulted from the limited number of individuals available.

In addition, individuals of *P. m. bairdii* (laboratory stock, University of Michigan), *P. leucopus* (feral animals from Washtenaw County, Michigan) and *P. polionotus* (laboratory stock, University of Michigan) were used for immunization, although the results herein reported are primarily concerned with the antigenic characteristics of individuals of the subspecies *P. m. gracilis* exhibited by means of anti- *P. m. gracilis* rabbit sera.

METHODS

The preparation of erythrocytes and sera, as well as typing methods, followed standard serological procedures (cf. Dunsford and Bowley, 1955). Blood was obtained from the mice by clipping the tail or by inserting a 1·5–2·0 mm. capillary tube into the suborbital canthal sinus. Approximately 0·5 c.c. of blood could be collected, and rarely were there fatalities.

All agglutination reactions were performed by the standard saline agglutinin tube test. Macroscopic readings of reactions were observed before and after centrifugation. The agglutinating reactions characterizing the A and B antigens here reported were easily defined, stable agglutinated masses of cells. In anticipation of the genetic data presented later, no differences were noted as to strength of positive reaction between heterozygote and homozygote positives. All typing series performed after initial identification of the antigens included known positive and negative cell types.

Heteroimmunization

In heteroimmunizing a rabbit with mouse erythrocytes an individual mouse was used for the complete immunization schedule of a rabbit. The initial injection consisted of a mixture of 0·5 c.c. Freund's (complete) bacto-adjuvant and 0·15 to 0·2 c.c. of washed, packed mouse erythrocytes introduced subcutaneously in the shoulder region of the rabbit. On the twenty-fourth and again on the twenty-fifth day following the subcutaneous injection, the rabbit was injected intravenously with a 50% suspension of erythrocytes from the same donor mouse. A total of 0·15 to 0·2 c.c. of packed cells was injected on those subsequent days.

Unabsorbed rabbit serum samples were periodically titred by the doubling dilution technique. The immune anti-mouse titre of unabsorbed rabbit sera rose to a final plateau in the range of 1:2000 to 1:4000 approximately 21 days following the subcutaneous injection, at which time final bleeding of the rabbit was performed.

Absorption

Multiple absorption analysis of immune rabbit sera was performed by standard procedures which are briefly summarized as follows. A total volume of approxi-

mately 0·1 c.c. of immune rabbit serum was absorbed stepwise through aliquots of erythrocytes (usually three) containing a total volume of 0·2 to 0·3 c.c. of packed cells from an individual mouse. The final reagents, exhibiting no agglutination reaction toward the absorbing cells, were tested for agglutinins against cells of other mice used for absorption of the immune sera.

Analysis of immune sera from three rabbits immunized with *P. m. gracilis* cells absorbed with individual cell samples of blood from forty-nine different mice showed patterns of absorption indicated by agglutination tests as summarized in Table 1. Cells were classified into three groups on the basis of absorption and reaction characteristics. The two reactive agglutinins are designated as anti-A and anti-B. Attempts by absorption to fractionate the anti-A and anti-B reagents were unsuccessful, and these two reagents are therefore considered to represent specific antibodies within the sera.

Table 1. *Summary of absorption analysis of heteroimmune anti-*Peromyscus maniculatus gracilis *sera with* P. m. gracilis *erythrocytes*

	Immune rabbit sera								
	anti-group 1			anti-group 2			anti-group 3		
	Types of absorbing cells								
Tested erythrocytes	1	2	3	1	2	3	1	2	3
Group 1 (type AB)	−	+	+	−	−	+	−	+	−
Group 2 (type A)	−	−	+	−	−	+	−	−	−
Group 3 (type B)	−	+	−	−	−	−	−	+	−

\+ = agglutination.
− = non-reactivity.

Absorption of the anti-AB serum with type A cells and, subsequently, with type B cells rendered the serum non-reactive with the donor type AB cells. These results exclude the existence of an interaction antigen, in this particular anti-AB serum, of the sort noted with some heterozygote serotypes (Cohen, 1960). The titres of the anti-A and anti-B reagents when tested by doubling dilution technique against cells from the immunizing or donor mouse were 1/64 and 1/32, and typing reactions were performed using 1/12 and 1/8 dilutions, respectively. Those dilutions gave strong diagnostic reactions. Following the original identification of each of the antibodies in various immune sera, a single rabbit serum was chosen for preparation of each of the reagents. A limited number of previously tested absorbing bloods were repeatedly used for preparation of typing reagents.

Judging from the absorption methods, two unitary saline (complete) agglutinins were isolated from the immune sera and each exhibits a different pattern of reactions toward erythrocytes of individual mice. All *P. m. gracilis* tested possessed either or both of the antigenic types; no doubly non-reactive mouse was observed in the 344 individuals of the laboratory stock tested.

Isoimmunization

Two methods of isoimmunization within the *P. m. gracilis* stock were used in an attempt to obtain isoimmune sera.

A series of three intraperitoneal injections, each of a 25% suspension of 0·1 to 0·2 c.c. packed erythrocytes, was used in a group of ten donor-recipient pairs. The injections were administered at 3-day intervals, and 7 days following the last injection the recipient mouse was bled into an uncitrated tube. The unabsorbed sera of the recipient bloods were tested for agglutination against erythrocytes of the donor mice and in all cases were negative. Subsequent typing of mice with immune rabbit sera revealed that six of the donor-recipient pairs of this group differed antigenically such that an immune response might have been expected.

Subsequent to the exhibition of differences by immune rabbit sera, nine new donor-recipient pairs of mice were used for isoimmunization; of these, five pairs consisted of serotype A donors and serotype B recipients and four pairs consisted of serotype B donors and serotype A recipients. The initial dose consisted of a subcutaneous injection of an emulsion of 0·05 to 0·1 c.c. of washed, packed erythrocytes and 0·2 c.c. of Freund's (complete) bacto-adjuvant. Twenty-two days later, 0·5 c.c. of a 20% erythrocyte suspension was injected intravenously into the ventral base of the tail, and six days thereafter the recipient mouse was bled and the unabsorbed serum tested for hemagglutination with donor and control cells.

In a doubling dilution series, in which each of the nine isoimmune sera were tested against erythrocytes from the donor individual, agglutinating titres were as follows: isoimmune anti-A sera, 1/64, 1/16, 1/8, 1/1 and a dilution greater than 1/1; isoimmune anti-B sera, 1/2048, 1/256, 1/128, 1/1.

Reactions of the unabsorbed isoimmune sera paralleled those of the immune rabbit sera and indicated that the isoimmune sera represented the same agglutination system as defined by the heteroimmune sera.

The success of the second procedure appears to indicate that a prolonged exposure (e.g. subcutaneous adjuvant emulsion) followed by a large direct circulatory injection of the antigen was necessary for the elicitation of an observable isoimmune response.

GENETIC BASIS OF ANTIGENIC POLYMORPHISM

A summary of mating types and observed offspring in the laboratory stock of *P. m. gracilis* is given in Table 2. In these tests of allelism of the two antigenic factors no sibship segregated for more than three phenotypes. Moreover, the data are consistent with the simplest hypothesis that the two antigenic types are controlled by two co-dominant alleles at a single locus.

On another hypothesis, namely that the two agglutinogens are determined by dominant genes at two separate loci, a mouse of the AB blood type should be identifiable as a double heterozygote, having the genotype X^A/x, Z^B/z, if it satisfies either of the following criteria: (1) it has one type A and one type B parent, or (2)

Table 2. *Summary of mating-offspring antigenic phenotypes in* Peromyscus maniculatus gracilis*

Antigenic phenotype of mating	Number of sib-ships	Total number of off-spring	Total offspring of various phenotypes				χ^2
			A	AB	B	Non-reactive	
1. A × A	2	15	15	0	0	0	—
2. B × B	2	12	0	0	12	0	—
3. A × B	10	57	0	57	0	0	—
4. A × AB	7	50	26	24	0	0	0·08, d.f. = 1, p = 0·75–0·90
5. B × AB	13	61	0	27	34	0	0·81, d.f. = 1, p = 0·25–0·50
6. AB × AB	18	127	38	55	34	0	2·29, d.f. = 2, p = 0·25–0·50
7. B × unknown	1	13	0	0	13	0	—
8. Unknown	9	9	2	4	3	0	—
Total	62	344					

Data of mating types numbers 7 and 8 have been included primarily to indicate the absence of a non-reactive type, and to indicate the total number of individuals with serologically untyped parentage contributing to the colony.

* All χ^2 tests are based on hypothesis of two-allele, one-locus mode of inheritance.

its offspring include mice exhibiting the three blood types, A, AB, and B. Assuming that the typed offspring are representative of the frequency of random gametic unions, one-sixteenth of the offspring from matings between two such double-heterozygotes are expected to exhibit a fourth, non-reactive, phenotype of the genotype x/x, z/z. The probability of not observing individuals lacking both antigens, therefore, becomes $(15/16)^n$, where n represents the total offspring observed in AB by AB matings ascertained by the above criteria as double heterozygotes. A total of 112 offspring of such matings was observed, thereby giving a probability of less than 0·001 with a two-locus model.

The breeding data indicate that the two antigens are determined by two co-dominant alleles, Pm^A and Pm^B.

In addition to the summary data, no individual pedigree of any mating (in some cases through seven generations) necessitated use of a two-locus model to explain the observed breeding results.

OCCURRENCE OF THE A, B ANTIGENS IN OTHER FORMS

The two antigens have been noted in both feral and laboratory individuals of *P. m. gracilis* from Alger County, Michigan, and in feral and laboratory stock of *P. m. bairdii* from Washtenaw County, Michigan.

Only the A antigen has been observed in all forty-eight individuals of a laboratory stock of the beach mouse, *Peromyscus polionotus*. The eight ancestral mating pairs of these animals were collected in 1951 from Marion County, Florida, by Paul G. Pearson. The stock has undergone considerable inbreeding due to the small size of the colony.

The anti-A reagent (at the typing dilution of 1/12) and anti-B reagent (at 1/8) caused no agglutination of (1) erythrocytes of the house mouse (*Mus musculus*), representing various laboratory strains—STOLI/Lw, C3H, SEC/Re, C57 BL/6, ST/JAX, (2) with human erythrocytes possessing the human A, B, M or N antigens, and (3) with cells of eleven mice of the species *Peromyscus leocupus* collected by Michael Petras and the author in Washtenaw County, Michigan.

OTHER ANTIGENS

During this study other agglutinins were noted which appeared as inter-specific agglutinins. The absorption of anti-*P. maniculatus* rabbit serum (either anti-*P. m. gracilis* or anti-*P. m. bairdii*) with *P. leucopus* cells resulted in an anti-*P. maniculatus* reagent which reacted with cells of all twelve (eight *P. m. gracilis*, four *P. m. bairdii*) individuals of *P. maniculatus* tested; the converse was also true (with all eight individuals of *P. leucopus* tested.) These findings are similar to those noted by Moody (1941). Although anti-*P. m. gracilis* serum when absorbed with cells from individuals of *P. m. gracilis* resulted in reagents which could be used to type individuals of *P. m. gracilis* or *P. m. bairdii* for the A and B antigens, the absorption of anti-*P. m. gracilis* sera with cells from type AB individuals of *P. m. bairdii* resulted in an anti-*P. m. gracilis* reagent reacting with cells of all twenty-four *P. m. gracilis* tested. The absorption of anti-*P. m. bairdii* sera with cells from type AB individuals of *P. m. gracilis* produced an anti-*P. m. bairdii* reagent reacting with the cells of all seven individuals of *P. m. bairdii* tested. These population antigenic differences are similar to those reported by Moody (1948), although the differences here reported were observed using erythrocytes from individual mice for absorption.

Anti-*Peromyscus polionotus* sera could not be fractionated by multiple absorption analysis using the laboratory stock of *P. polionotus* previously mentioned.

The results indicate that absorption and immunization procedures using samples of erythrocytes from individuals of small mammals are feasible for the isolation and identification of antigenic characteristics dependent upon simple genetic systems. Such genetic characters should be a valuable aid in the study and analysis of various problems of polymorphisms in populations of small mammals.

SUMMARY

1. Antigenic polymorphism between individuals of the subspecies *Peromyscus maniculatus gracilis* is demonstrated.

2. Absorption analysis of heteroimmune rabbit sera using erythrocytes of individual mice revealed two unitary complete (saline) agglutinins, designated as anti-A and anti-B, reactive to erythrocytic antigens in this species.

Isoimmunization apparently demonstrated these same antigenic differences.

3. The two antigenic characters (A and B) defined by the agglutinins are inherited as if simply related to allelic factors designated Pm^A and Pm^B respectively.

4. These two antigens are found in representatives of both the subspecies *P. m. gracilis* and *P. m. bairdii*, and, in addition, the A antigen was observed in a

laboratory stock of *P. polionotus*. Tested individuals of the species *P. leucopus* and *Mus musculus*, however, possessed neither antigen, and samples of human blood also lacked both antigens.

5. Species-specific agglutinins distinguishing individuals of the species *P. leucopus* and *P. maniculatus*, and population-specific agglutinins which distinguished between individuals of the subspecies *P. m. bairdii* and *P. m. gracilis*, have been observed.

I extend my gratitude to the following staff members of the University of Michigan: Dr Morris Foster and Dr Elizabeth Barto of the Department of Zoology, for their constant concern and invaluable aid during the study. For encouragement and erudite advice concerning serological procedures I thank Dr Henry Gershowitz, Department of Human Genetics. Each of the above, as well as Dr E. T. Hooper, Department of Zoology, and Dr J. N. Spuhler, Department of Anthropology, made valuable suggestions and criticisms concerning preparation of the manuscript.

REFERENCES

Cohen, C. (1960). A second example of a rabbit red cell antigen resulting from interaction of two genes. *J. Immunol.* **84**, 501–506.

Cotterman, C. W. (1944). Serological differences in the genus *Peromyscus* demonstrable with normal human sera. *Contr. Lab. Vertebr. Biol. Univ. Mich.* **29**, 1–13.

Dunsford, I. & Bowley, C. C. (1955). *Techniques in Blood Grouping*. C. C. Thomas, Springfield, Ill.

Gorer, P. A. (1936). The detection of antigenic differences in mouse erythrocytes by the employment of immune sera. *Brit. J. exp. Path.* **17**, 42–50.

Moody, P. A. (1941). Identification of mice in the genus *Peromyscus* by a red cell agglutination test. *J. Mammal.* **22**, 40–47.

Moody, P. A. (1948). Cellular antigens in three stocks of *Peromyscus maniculatus* from the Columbia River Valley. *Contr. Lab. Vertebr. Biol. Univ. Mich.* **39**, 1–16.

Genet. Res., Camb. (1961), **2**, *pp.* 456–460
Printed in Great Britain

Polygene analysis

II. SELECTION

BY NEIL GILBERT

John Innes Institute, Bayfordbury, Hertford, Herts.

(*Received* 1 *May* 1961)

This paper continues and concludes a previous one (Gilbert, 1961).

CORRECTION

The suggestion on page 103 of the previous paper that the F_3 mean can provide extra information (when using the curved model) is wrong, since the F_3 mean is still linearly related to the F_1 and F_2 means ($2F_3 = 3F_2 - F_1$). This means that (1) the curved model is not sufficiently general and (2) a scaling test that compares the F_3 mean with the means of previous generations is no guarantee of additivity. This mistake does not affect the main argument; I apologize for my fallibility.

SELECTION

I shall consider the progress of selecting and selfing, starting from an F_2 progeny. The notation is that of the previous paper. A genotype homozygous for l 'good' genes, n 'bad', and heterozygous at m loci is referred to as (l, m, n). Its frequency in F_r is

$$\frac{(l+m+n)!}{l!\,m!\,n!} u_r^l v_r^m w_r^n \qquad (u+v+w = 1),$$

and it experiences a selection pressure $\alpha^l(2\beta)^m\gamma^n$. The relative values of $\alpha:\beta:\gamma$ are constant, but their absolute values in each generation are adjusted so that

$$u\alpha + 2v\beta + w\gamma = 1.$$

This form of selection pressure is chosen for its algebraic tractability; it implies that the selection pressures at different loci are the same, and are independent of each other. Then consideration of the frequency with which (l, m, n) is derived in F_{r+1} from each individual in F_r leads to the relations

$$\begin{aligned} u_{r+1} &= \alpha u_r + \tfrac{1}{2}\beta v_r \\ v_{r+1} &= \beta v_r \\ w_{r+1} &= \gamma w_r + \tfrac{1}{2}\beta v_r \end{aligned}$$

The implication—that the distribution remains multinomial in succeeding generations—is true for the form of selection pressure adopted. Since in F_2, $u = \frac{1}{2}v = w = \frac{1}{4}$, we find that, in F_{r+1},

$$u:v:w = \frac{\alpha^r - \beta^r}{\alpha - \beta} : 2\beta^{r-1} : \frac{\gamma^r - \beta^r}{\gamma - \beta}$$

these expressions being suitably multiplied to make their sum unity. This result can easily be checked by substitution in the above equations. It applies to *genotypic* selection from selfed progenies. Here I shall not consider other mating systems, nor the difficult problem of relating phenotypic to genotypic selection. At present, this result is useful only (1) for considering the possible importance, after selection, of the departures from additivity considered in the previous paper, and (2) as a theoretical basis for comparing responses to different rates of selection. (It is astonishing how rare are experiments to compare several different selection rates.) In the absence of selection, $\alpha = 2\beta = \gamma = 1$, so that $u = w = \frac{1}{2} - (\frac{1}{2})^{r+1}$ and $v = (\frac{1}{2})^r$.

If we adopt the curved model considered in the previous paper, the generation mean is approximately

$$\overline{F}_{r+1} = x^y[b + d_2 y + 4(1 - \overline{u-w}^2)d_1 + 2v(h_1 - 2d_1 + h_2 y)],$$

where

$$y = 2\frac{f+g}{f-g}(u-w).$$

This expression is obtained simply (if laboriously) by averaging the phenotype $[b + (l-n)d + mh]\,\theta^{l-n}$ over the multinomial distribution in l, m, n, and then substituting and approximating in θ in the manner of the previous paper. The expression is not symmetric in α and γ, so that upwards and downwards selection will give asymmetric results. If there is no selection,

$$\overline{F}_{r+1} = b + 4d_1 + (\tfrac{1}{2})^{r-1}(h_1 - 2d_1).$$

The recurrence relation $2\overline{F}_{r+1} = 3\overline{F}_r - \overline{F}_{r-1}$ then holds for the curved model, and the limiting value of $\overline{F}_r$ (as inbreeding proceeds) is $b + 4d_1$ which is equal to $2\overline{F}_2 - \overline{F}_1$. Here d_1 is a 'curvature' term which does not appear in the additive theory. On this theory, therefore, $2\overline{F}_2 - \overline{F}_1$ would be a better predictor than $\frac{1}{2}(P_1 + P_2)$ of the average result of unselected inbreeding. Finally, the limit of upward selection ($u = 1$, $v = w = 0$) is $x^y(b + d_2 y)$, where y is now $2(f+g)/(f-g)$. This result could of course have been obtained directly from the original expression for the phenotype of $(f+g, 0, 0)$. It is necessary, for the argument of the next section, to note that y is a function of the degree of genetic dissimilarity between the two parents; it is independent of the genotype-phenotype relation.

SCALING TESTS

Giesbrecht (1961) has published some more sets of family means. To fit the expressions given on page 101 of the previous paper to six means, we must solve the quartic

$$\phi(x) = x^2(P_2 x^2 - 2B_2 x + F_1) - 2x(B_2 x^2 - 2F_2 x + B_1) + (F_1 x^2 - 2B_1 x + P_1) = 0$$

(obtained by eliminating b, d_1, d_2, h_1, h_2). In no less than five of Giesbrecht's six examples, this equation has no real root near $x = 1$. The diploid inheritance of the characters concerned cannot be doubted. The curved model therefore cannot be fitted to Giesbrecht's figures. In the first paragraph we saw that the curved model

imposed a relation between the F_3 mean and the F_1 and F_2 means, and concluded that the model is insufficiently general. The same conclusion must obviously be drawn here. Now it is perfectly simple to generalize the model further, perhaps by including a multiplicative dominance term so that the phenotype of (l, m, n) becomes $[b+(l-n)d+mh]\theta^{l-n}\psi^m$. But there are then too many parameters to be fitted to a limited set of data; statistically speaking, the situation is hopelessly fluid. Now this unfortunate result is not due merely to the unavoidable errors of estimation of family means, nor is it entirely due to the vagaries of the genotypic-phenotypic relation; to some extent it is intrinsic in the genetic relationship between inbred parents and their offspring. To see this, we consider a hypothetical case wherein the family means are known exactly and the three scaling tests

$$P_2-2B_2+F_1 = B_2-2F_2+B_1 = F_1-2B_1+P_1 = 0$$

are satisfied, so that $x = 1$ is a solution of $\phi(x) = 0$. But

$$\frac{1}{4}\frac{d\phi}{dx} = x(P_2x^2-2B_2x+F_1)-(B_2x^2-2F_2x+B_1),$$

so that the scaling tests ensure, not only that $\phi = 0$ at $x = 1$, but also that $d\phi/dx = 0$. Consequently, any arbitrary value of x in the neighbourhood of 1 will fit the data. I take the $D\times J$ 1938 data used by Mather (1949) as an example, because they most nearly satisfy the scaling tests; other data that satisfy these tests will give similar results. Take arbitrary values 0·9, 1, 1·1 for x, we find:

x	0·9	1	1·1	
b	7·86	7·31	7·09	(±0·06)
d_1	−0·03	0·10	0·16	(±0·05)
d_2	1·71	0·91	0·23	(±0·03)
h_1	−1·17	−0·90	−0·79	(±0·05)
h_2	−0·05	0·05	0·11	(±0·07)
Σ(obs.−exp.)^2	0·0019	0·0019	0·0012	

The figures in brackets are the standard errors of the various parameters. Σ(obs.−exp.)^2, the sum of squares of deviations of the family means from their expectations, is quoted here merely to indicate that—as is easily verified—the different values of x give (roughly) equally good fits. It may be objected that the additive and multiplicative terms in the model are compensating each other. This is true as far as the family means are concerned. But when we extrapolate outside the range from P_1 to P_2, the consequences are quite different. Taking arbitrary (but plausible) values for y, the limit of selective advance becomes:

$x=$	0·9	1	1·1	
		$x^y(b+d_2y)$		
$y=2$	9·13	9·13	9·13	$(=P_1)$
3	9·46	10·04	10·35	
4	9·64	10·95	11·72	
5	9·68	11·87	13·26	

We must conclude that the scaling tests do not guarantee additivity; they guarantee an indeterminate situation in which additivity is one solution. Of course, it is always possible to draw an infinite number of curves through a finite number of fixed points, but if these points are collinear the curves must usually appear improbably tortuous in comparison with the straight line. The present case is peculiar in that there is no apparent reason to prefer the additive solution, for the alternative models seem perfectly reasonable. One can of course invoke Occam's Razor to argue that, if the system admits an additive interpretation, it should be additive. But there is no guarantee that the genes will agree.

It follows, then, that predictions of absolute advance under selection—based on polygene analysis—cannot be relied on. (They may, of course, chance to be accurate in some cases; but we do not know which cases these will be.) This practical failure of polygene analysis does not invalidate the theoretical side of polygene work. Such ideas as that of polygene balance (Mather, 1943) are very valuable. But the practical difficulties are legion. In the previous paper it was shown that great care is needed in the choice of scale, before the additive analysis can be used. Worse remains behind; for it now appears that the assumption of additive gene effects would still be only an assumption, even if the scaling tests were perfectly satisfied. As soon as we investigate a simple non-additive model, the situation becomes indeterminate. For some things (e.g. the heritability) this may not matter much; for others (response to selection) it clearly does. I have not found a way of resolving this difficulty. The example above also shows that dominance and curvature are to some extent confounded. For practical analysis of quantitative inheritance, it seems wiser to adopt simpler methods, e.g. heritability, combining ability, offspring-parent regression, or the use of marker genes. It is particularly unfortunate that polygene analysis should sometimes be advocated as an aid to practical breeding work. Of course, the simpler methods mentioned also involve an additive hypothesis, and so might be equally sensitive to failure of additivity. But they might not; for the unpretentious model underlying the 'heritability' of an outbreeding population is almost a statistical convention in comparison with the detailed genetic assumptions involved in the polygene analysis of inbred parents. On the other hand, elaboration of the 'heritability' model to include terms for dominance, epistacy, etc., is likely to suffer, once again, from the kind of curvature discussed in this paper. (It is doubtful whether such elaboration is very helpful or meaningful, anyway.) This paper shows that polygene analysis has an intrinsic theoretical weakness. The curved model of gene action, being more flexible than the strictly additive model, shows up this weakness admirably; but neither the additive nor the curved model is sufficiently flexible to describe real life.

SUMMARY

A modest theory of polygene selection is presented. Recently published data show that the curved model of gene action (presented in a previous paper) is insufficiently general. The curved model does, however, show that the scaling tests (used in the additive type of polygene analysis) do not guarantee additivity; they guaran-

tee a state of indeterminacy, in which additivity is one of a range of reasonable possibilities. These different possibilities give entirely different predictions of selective advance. The failure (in practice) of polygene analysis does not reduce the value of polygene concepts.

I wish to thank the Referee for his valuable suggestions.

REFERENCES

GIESBRECHT, J. (1961). The inheritance of ear height in *Zea mays*. *Canad. J. Genet. Cytol.* **3**, 26–33.

GILBERT, N. (1961). Polygene analysis. *Genet. Res.* **2**, 96–105.

MATHER, K. (1943). Polygenic inheritance and natural selection. *Biol. Rev.* **18**, 32–64.

MATHER, K. (1949). *Biometrical Genetics*. London: Methuen.

VOLUME II

INDEX OF SUBJECTS AND AUTHORS

Only main topics are listed, and reference is given to the first page of each paper in which they are discussed

Abplanalp, H., 439
Anderson, G. E., 414
Antigenic differences, in erythrocytes of *Peromyscus*, 449
Auerbach, C., 63

Beale, G. H., 82
Boam, T. B., 161
Body size, inheritance in mouse, 25
Burnet, B., 249

Carter, T. C., 296
Cattanach, B. M., 156
Chai, C. K., 25
Chromosome
  polymorphism in *Drosophila subobscura*, 1
  substitution, effect on growth in *Drosophila*, 346
  unbalanced constitutions in mouse, 156
  variation of number in *Rubus* hybrid, 10
Clarke, J. M., 70
Coelopa frigida
  ecology and habitat, 249
  embryonic lethals in natural populations, 249
Cohen, B. L., 306
Combining ability for yield in tomato, 361
Complementation between methionine suppressors in *Coprinus*, 141
Coprinus lagopus
  linkage in, 414
  methionine supressors in, 141
Correlation between minor skeletal variants in mouse, 431
Crossing-over, induced mitotic, in *Ustilago*, 231
Cytology, meiosis of F_1 *Oryza* hybrid, 373

Day, P. R., 414
Development
  of chromosomal number in *Rubus* hybrid, 10
  of 'Oligosyndactylism' in mouse, 33
  of 'pudgy' in mouse, 384
  selection for rate of, in *Drosophila*, 70
Dhaliwal, S. S., 309
Drosophila melanogaster
  ecological genetics of growth, 346, 424
  selection for sternopleural chaetae number, 161
  selection for sternopleural chaetae pattern, 158
  sensitivity of testis to TEM, 63
Drosophila subobscura
  chromosomal polymorphism, 1
  selection for rate of development, 70

Edwards, R. G., 272
Environment
  aseptic synthetic diets for *Drosophila*, 346, 424
  different temperatures for *Drosophila*, 424
Escherichia coli
  attachment of *F* factor to specific chromosomal region, 333
  episomic alternation, 333
  genetics of fimbriation, 394
  negative interference in, 406
Evolution
  natural selection accumulating genetic information, 127
  of *Oryza* spp., 373

Fimbriation, genetics of, in *Escherichia*, 394
Fowler, R. E., 272
Frost, L. C., 43

Gene frequency, of embryonic lethals, in *Coelopa*, 249
Genetic information in evolution, 127
Genetic models
  for changes in state of gene, 333
  natural selection in evolution, 127
  of negative interference, 406
  polygene analysis, 96, 456
  responses to selection, 70
Genetic parameters
  estimates from progeny tests, 195
  linear estimates of, 439
Gibson, I., 82
Gilbert, N., 96, 361, 456
Growth, ecological genetics of, in *Drosophila*, 346, 424
Grüneberg, H., 33, 384

Haskell, G., 10
Hayes, W., 394, 406
Heritability
  estimates from progeny tests, 195
  linear estimates of, 439
Heterosis of yield in tomato, 361
Histology of 'bare' mutant in mouse, 283
Holliday, R., 204, 231
Homeostasis, of growth and development in *Drosophila* F_1's, 424

Inbreeding during selection, 189
Interaction
  between growth genes in *Drosophila*, 346
  combining ability in yield of tomato, 361
  gene-environment, in *Drosophila*, 346, 424
Inversions in *Drosophila subobscura*, 1

Kimura, M., 127
Knight, G. R., 1
Kojima, K., 177

Lethals, recessive embryonic, in *Coelopa*, 249
Lewis, D., 141
Linkage
  in *Coprinus*, 414
  in *Ustilago*, 204
  of 'dreher' in mouse, 92
  of 'tipsy' in mouse, 122
Lyon, M. F., 92, 296

Maccacaro, G. A., 394, 406
Mate-killer trait, genetic basis of, in *Paramecium*, 82
Maynard Smith, J., 70
Meiosis of F_1 *Oryza* hybrid, 373
Mouse
  correlations between minor skeletal variants, 431
  description and linkage of 'tipsy', 122
  development of 'Oligosyndactylism', 33
  genetics and development of 'pudgy', 384
  induction of mutations by X-rays, 296
  inheritance of body size, 25
  linkage and pleiotropy of 'dreher', 92
  mutants
    'bare', recessive hairlessness, 283
    'Dappled', sex-linked dominant coat variegation, 290
    'midget' recessive dwarfing, 272
    'Oligosyndactylism', dominant digit fusion, 33
    'pudgy', recessive skeletal malformation, 384
    'tipsy', recessive locomotory disorder, 122
Mouse—*continued*
  mutations at histocompatibility loci, 309
  transferrin locus, revised nomenclature, 306
  unbalanced sex-chromosome constitutions, 156
Mutants
  'bare', recessive hairlessness in mouse, 283
  'Dappled', dominant coat variegation in mouse, 290
  'dreher', recessive locomotory disorder in mouse, 92
  'midget', recessive dwarfing in mouse, 272
  'Oligosyndactylism', dominant digit fusion in mouse, 33
  'pudgy', recessive skeletal malformation in mouse, 384
  'tipsy', recessive locomotory disorder in mouse, 122
  biochemical, in *Ustilago*, 204
  methionine, in *Coprinus*, 145
Mutation
  induced by TEM, in *Drosophila*, 63
  induced by X-rays, in mouse, 296
  of *F* factor, in *Escherichia*, 333
  of histocompatibility loci, in mouse, 309

Natural selection, accumulating genetic information, 127
Negative interference
  fimbriation in *Escherichia*, 394
  models of, 406
Neurospora crassa, recombination frequencies, 43
Non-disjunction of sex-chromosomes in mouse, 156

Oryza spp., meiosis of F_1 hybrid, 373

Paramecium aurelia, mate-killer trait, 82
Peromyscus maniculatus, erythrocytic antigenic differences in, 449
Phillips, R. J. S., 290
Pleiotropic effect of 'dreher' in mouse, 92
Polygene analysis, 96, 456
Polygenic inheritance of body size in mouse, 25
Polymorphism
  antigenic, in *Peromyscus*, 449
  chromosomal, in *Drosophila*, 1
Population size, effective, during selection, 189
Poultry, estimates of genetic parameters, 439
Prabhu, S. S., 424
Progeny tests for heritability estimates, 195

Radiation, induced mutation by
of recessive lethal and visible mutations in mouse, 296
of histocompatibility loci in mouse, 309
Randelia, H. P., 283
Rao, D. R. R., 373
Rasmussen, D. I., 449
Recombination frequencies, heterogeneity of, in *Neurospora*, 43
Reddi, O. S., 63
Reeve, E. C. R., 158, 195
Richter, A., 333
Robertson, A., 189
Robertson, F. W., 346, 424
Rubus hybrid, variation in chromosome number, 10

Sanghvi, L. D., 283
Scaling tests for polygene analysis, 96, 456
Searle, A. G., 122
Selection
efficiency of various methods, 106
for body size in *Drosophila*, 346
for rate of development in *Drosophila*, 70
for sternopleural chaetae number in *Drosophila*, 161
for sternopleural hair pattern in *Drosophila*, 158
for yield in tomato, 361
inbreeding during, 189
limits and further response to, 161
model of response to, 456
theory of, in small populations, 177
Shastry, S. V. S., 373
Shreffler, D. C., 306
Skeletal abnormalities
correlations between minor variants, in mouse, 431
Skeletal abnormalities—*continued*
of 'Oligosyndactylism' mutant, in mouse, 33
of 'pudgy' mutant, in mouse, 384
Sondhi, K. C., 70
Spermatogenesis, sensitivity to TEM, in *Drosophila*, 63
Sternopleural chaeta
selection for number of, in *Drosophila*, 161
selection for pattern of, in *Drosophila*, 158
Substitutional load, 127
Suppressors, of methionine mutants, in *Coprinus*, 141

Techniques of *Ustilago* culture, 204
TEM
induced mutation by, of histocompatibility loci in mouse, 309
sensitivity of *Drosophila* testis to, 63
Tetrad analysis in *Ustilago*, 204
Thoday, J. M., 161
Tomato, selection for yield in, 361
Transferrin locus in mouse, revised nomenclature of, 306
Truslove, G. M., 431
Tumour cells, histocompatibility mutations in mouse, 309
Tun, N. N., 10

Ustilago maydis
genetics of, 204
mitotic crossing-over in, 231

Variance of heritability estimates, 195

Weight, inheritance of, in mouse, 25

Young, S. S. Y., 106